

## Endoplasmic Reticulum Protein Processing Is a Molecular Target of Ginsenoside Rk1 in Cervical Cancer Cells

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### ABSTRACT

Modifications in work-life balance have contributed to higher rates of cervical cancer in younger adults. Ginsenoside Rk1, a low-abundance saponin component of ginseng, is recognized for its capacity to restrain growth and survival across multiple human cancer cell types. Nonetheless, its influence on the proliferative capacity of HeLa cells has not been established. Exposure to ginsenoside Rk1 caused HeLa cells to arrest in the G0/G1 phase in a manner dependent on concentration, while also suppressing subsequent cell division and expansion. The agent robustly engaged the apoptotic machinery, involving key mediators such as caspase 3, PARP, and caspase 6. It further upregulated LC3B protein abundance, pointing to enhanced autophagic activity. Integrated Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses identified pronounced downregulation of the protein processing in the endoplasmic reticulum pathway. These patterns were confirmed through real-time quantitative PCR and western blot analyses, which documented sharp reductions in the levels of YOD1, HSPA4L, DNAJC3, and HSP90AA1 transcripts and proteins in treated cells. YOD1 displayed the most substantial decline in response to ginsenoside Rk1. The observed cytotoxicity of ginsenoside Rk1 toward HeLa cells can be attributed to interference with endoplasmic reticulum-based protein maturation combined with amplified apoptotic signaling. These mechanisms highlight YOD1 as a candidate target for future cervical cancer interventions.

**Keywords:** Ginsenoside Rk1, Cervical cancer, Protein processing in endoplasmic reticulum, RNA seq

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### Introduction

Cervical cancer ranks among the leading gynecologic malignancies, most often appearing in women 35–55 years old. However, incidence has risen noticeably in younger cohorts due to evolving daily routines and workplace demands [1]. Studies have established strong ties between HeLa cells, persistent human papillomavirus infection, and impaired immune surveillance [2]. Because early signs are frequently nonspecific, diagnosis commonly occurs at late stages, limiting surgical options and shifting treatment toward chemotherapeutic agents aimed at destroying malignant cells [3].

Ginseng displays broad therapeutic potential, including anti-aging [4], antitumor, pain-relieving [5], mood-stabilizing, and immune-enhancing activities [6]. Its dominant bioactive fraction consists of ginsenosides, which have attracted considerable scientific attention. Existing reports indicate that Rk1 lowers telomerase function, hinders cellular expansion, and triggers visible structural disruptions in cancer cells. The linkage between telomerase blockade and programmed cell death is thought to drive much of Rk1's anticancer efficacy [7]. Whether this compound can similarly limit cervical cancer cell multiplication, however, requires further clarification.

Classical experimental strategies for investigating tumor proliferation depend on targeted biochemical and molecular assays that are time-consuming and offer restricted scale. RNA sequencing (RNA-seq), by sequencing cDNA libraries prepared from cellular RNA, provides a high-throughput means to map gene activity comprehensively [8]. In the present work, ginsenoside Rk1 potently curbed proliferation and elicited apoptosis in cultured cancer cells. Comprehensive RNA-seq profiling uncovered widespread changes in gene networks and pathways after compound administration. Validation experiments employing western blotting and real-time quantitative PCR (RT-qPCR) delineated the central mechanisms through which ginsenoside Rk1 exerts control over HeLa cell fate, reinforcing its promise as an agent capable of halting growth and driving cell death.

## Materials and Methods

### *Materials and instruments*

Phosphate-buffered saline (PBS), CCK-8 kit, cell cycle analysis kit, CHCl<sub>3</sub>, nuclease-free sterile water, and apoptosis assay kit were obtained from Beijing Suo Laibao Technology Co., Ltd. Ginsenoside Rk1 was supplied by Jinsheng Biotechnology Co. Ltd. Antibodies directed against caspase 3, PARP, caspase 6, LC3B, YOD1, HSPA4L, DNAJC3, and HSP90AA1 were purchased from Beijing Boasen Biological Co. Ltd. Fetal bovine serum (FBS) came from Beijing Baiao Laibo Technology Co., Ltd., and PVDF membranes were from Millipore. Reverse transcriptase (1896649) and R0133DUTP were provided by Invitrogen. SuperScript™ II Reverse Transcriptase, m0209, and Escherichia coli DNA polymerase I were acquired from Ambion. The AHTS mRNA-seq library construction kit and phenol-chloroform reagents originated from Thermo Fisher Technology Co., Ltd. (China). Nucleic acid cleanup kits were sourced from Aide Technology Co., Ltd., while the one-step SYBR Green qPCR master mix was from Yeasen. All additional reagents met analytical purity standards. Equipment support was furnished by the new food resources processing and application group.

### *Cell resuscitation and culture*

HeLa cells were obtained from Delta Biotechnology Co., Ltd. Frozen stocks were thawed and gently pipetted to produce uniform single-cell suspensions. These were plated and maintained in a humidified incubator at 37°C with 5% CO<sub>2</sub> using established culture conditions [9].

### *Inhibitory effects of ginsenoside Rk1 on HeLa cells*

Cells were seeded into 96-well plates at  $1 \times 10^5$  cells per well and incubated for 24 h under standard conditions (37°C, 5% CO<sub>2</sub>). They were then treated for 24 h with serial concentrations of ginsenoside Rk1 (1, 2, 4, 8, 10, 20, and 30 μM). Ten microliters of CCK-8 reagent were added to each well, followed by 1 h incubation at 37°C in darkness. Absorbance values at 450 nm were recorded on a microplate spectrophotometer. Percentage viability was computed via the formula:

$$\text{Cellviability(\%)} = (\text{OD}[\text{Rk1 group}] - \text{OD}[\text{blank group}]) / (\text{OD}[\text{control}] - \text{OD}[\text{blank group}]) \times 100\% \quad (1)$$

### *Apoptosis detection*

Programmed cell death was evaluated utilizing the Hoechst staining kit. After treatment, cells were fixed with 4% paraformaldehyde for 2 hours, and the fixative was subsequently discarded. Samples were then washed with phosphate-buffered saline (PBS) for 3 minutes. Staining was performed by incubating the cells with Hoechst 33258 for 5 minutes, followed by visualization of nuclear morphology under a fluorescence microscope. HeLa cells exposed to 10, 20, or 30 μM ginsenoside Rk1 were collected after digestion with 0.25% EDTA-free trypsin and washed using pre-chilled PBS. Flow cytometric analysis of apoptosis was conducted with Annexin V labeled by fluorescein isothiocyanate (Annexin V-FITC) serving as the fluorescent indicator.

### *Differential gene expression*

Selection of differentially expressed mRNAs relied on criteria of fold change exceeding 2 or falling below 0.5, together with a statistical significance threshold of  $p < 0.05$ , as determined through the edgeR package in R.

### *Gene Ontology and pathway enrichment analyses*

Functional classification, GO term assignment, and KEGG pathway mapping for the differentially expressed genes (DEGs) were executed via the Kobas 2.0 online server. The GO analysis incorporated the standard three domains: molecular functions, cellular components, and biological processes.

Pathway enrichment was assessed by applying a hypergeometric test to the KEGG database, treating individual pathways as units. This approach identified those pathways displaying statistically notable overrepresentation among the DEGs in comparison with the complete genomic background.

### RT-qPCR

Total RNA isolation and purification were carried out with TRIzol reagent as specified by the supplier. RNA yield and purity were measured on a NanoDrop ND-1000 spectrophotometer. Integrity was evaluated using an Agilent Bioanalyzer 2100 instrument (requiring RIN values above 7.0) and additionally confirmed via denaturing agarose gel electrophoresis. From 1 µg of total RNA, poly(A) RNA was enriched through two successive purification steps with Dynabeads Oligo(dT)25. Fragmentation of the poly(A) RNA occurred at 94°C for 5–7 minutes using the Magnesium RNA Fragmentation Module. After fragmentation, the material was reverse transcribed to cDNA and diluted ten-fold in RNase-free dH<sub>2</sub>O. The qPCR mixture contained 10 µL SYBR qPCR mix, 1 µL cDNA, 1.6 µL primer mix, along with appropriate volumes of forward and reverse primers (10 µL each), 80 µL RNase-free dH<sub>2</sub>O, and 7.4 µL enzyme-free water. Gene expression levels were normalized and quantified by the  $2^{-\Delta\Delta CT}$  method, with each sample assayed in triplicate.

### Western blotting analysis

Cells were harvested via trypsin treatment and completely lysed in cold lysis buffer at 4°C. Following centrifugation of the lysates at 12,000 rpm for 15 minutes at 4°C, the clear supernatant was collected and its protein content quantified with a BCA protein assay kit. Samples were combined with 10 µL of 12% SDS, boiled for 5 minutes, and separated by SDS-PAGE. PVDF membranes were washed with PBST for 5 minutes and then blocked in 5% non-fat milk prepared in TBST for 1.5 hours. After further PBST rinsing, membranes were probed overnight at 4°C in a cold chamber with primary antibodies against caspase 3, caspase 6, PARP, LC3B, or GAPDH (each at 1:1,000 dilution). Incubation with horseradish peroxidase-linked secondary antibody (1:10,000) was performed at 37°C for 1.5 hours. Signal detection was achieved using an ECL chemiluminescence kit, with images captured on a multi-function gel imaging system.

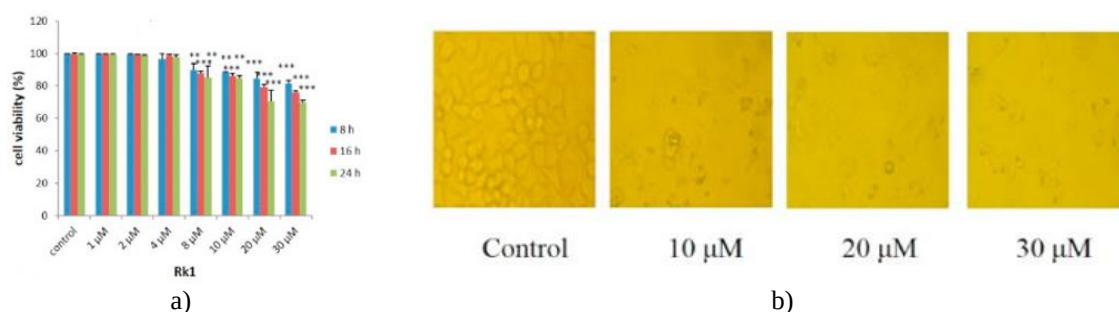
### Statistical analyses

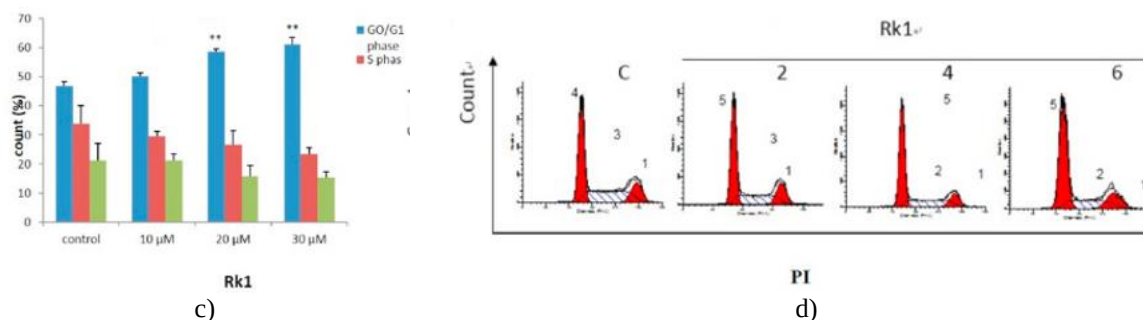
Experiments were independently replicated a minimum of three times, with results expressed as mean ± SD. Data processing and statistical testing were conducted in Origin 8.5 and Microsoft Excel. Group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance was defined as  $p < 0.05$  [10].

## Results and Discussion

### Inhibitory effects of ginsenoside Rk1 on HeLa cell growth

Exposure of HeLa cells to ginsenoside Rk1 over periods of 8, 16, and 24 hours revealed clear dose- and time-dependent suppression of cell proliferation (**Figure 1a**). At the 24-hour time point, the percentage inhibition of growth escalated from 15.1% to 30.3% when the concentration of ginsenoside Rk1 was raised from 10 µM to 30 µM.





**Figure 1.** Influence of ginsenoside Rk1 on HeLa cells. (a) Progressive inhibition of HeLa cell growth occurs with rising doses and longer treatment times of ginsenoside Rk1. (b) Morphological alterations in HeLa cells induced by ginsenoside Rk1. The saponin effectively limits malignant cell expansion. (c) and (d) Modulation of cell cycle progression in HeLa cells by ginsenoside Rk1. The compound restricts proliferation through blockade of cell division, thereby lowering overall proliferative potential. Relative to the untreated control, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

#### Effects of ginsenoside Rk1 on HeLa cell morphology

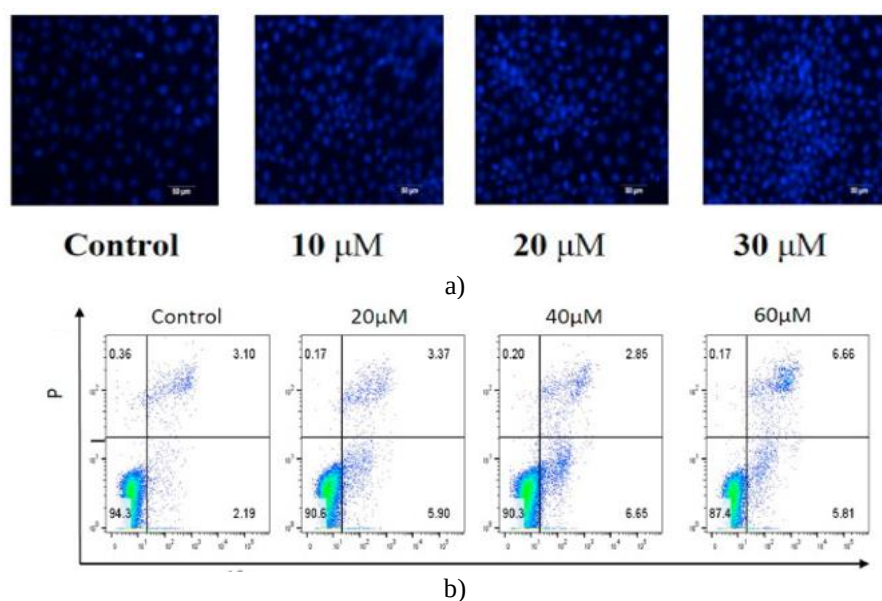
Untreated control HeLa cells displayed healthy morphology with minimal signs of apoptosis, presenting as uniform, intact structures possessing sharp boundaries (**Figure 1b**). In contrast, exposure to ginsenoside Rk1 at increasing concentrations produced distinct morphological transformations, such as cellular contraction, peripheral condensation, and notable size reduction, all hallmarks of apoptosis. Cell numbers declined dramatically when concentrations surpassed 30 μM. These morphological observations indicate that Rk1 promotes apoptotic processes, consistent with effects seen for related ginsenosides like Rh1.

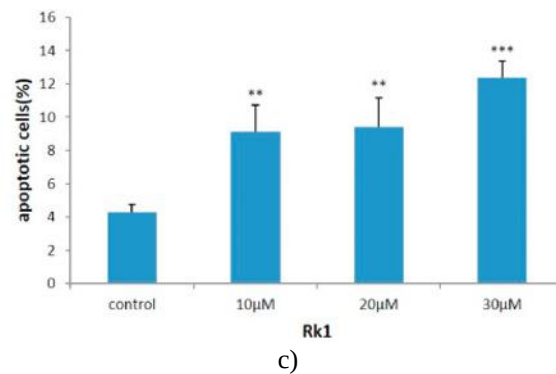
#### Effects of ginsenoside Rk1 on HeLa cell cycle

Application of ginsenoside Rk1 resulted in progressive, concentration-dependent accumulation of HeLa cells within the G0/G1 phase (**Figures 1c and 1d**). Notably, 20 μM and 30 μM doses caused significant elevation of G0/G1 cell populations ( $p < 0.01$ ) while simultaneously decreasing the fractions of cells in S and G2/M phases. Such findings establish that ginsenoside Rk1 disrupts normal cell cycle advancement, thereby retarding tumor expansion and attenuating proliferative activity.

#### Effects of ginsenoside Rk1 on HeLa cell apoptosis

Apoptotic responses in HeLa cells were investigated by monitoring morphological shifts in cultures with and without ginsenoside Rk1 exposure (**Figure 2a**). Treated cells exhibited characteristic apoptotic features, including chromatin condensation, intensely fluorescent bright blue nuclei, and abundant apoptotic fragments.





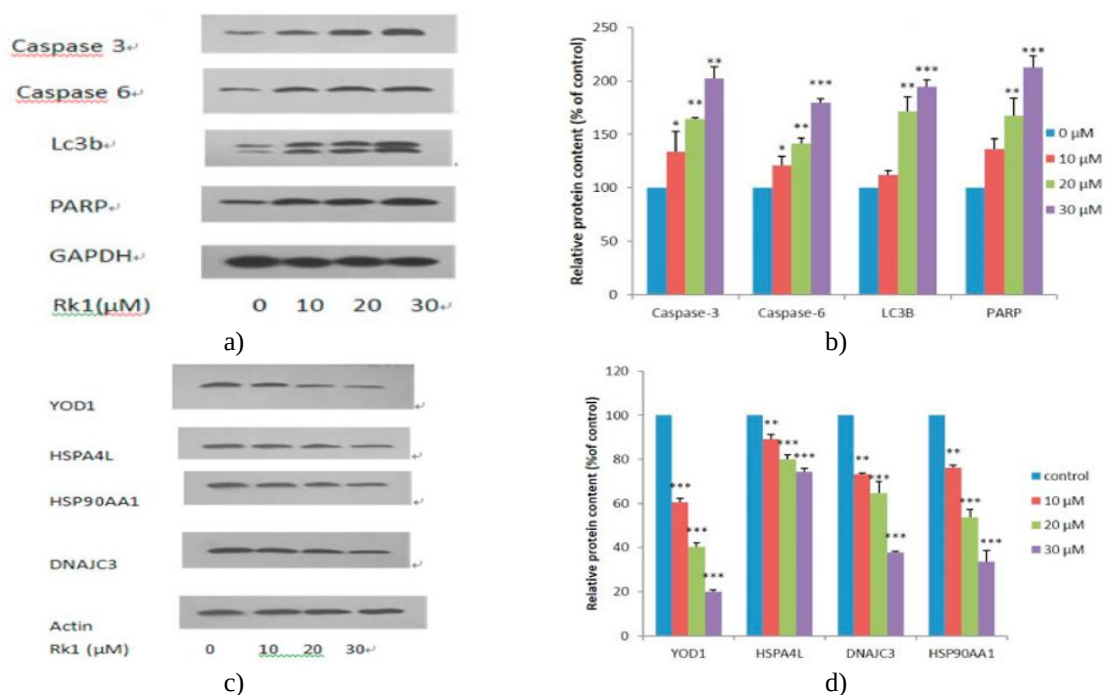
**Figure 2.** Effects of ginsenoside Rk1 on HeLa cell apoptosis. (a) Morphological evaluation of apoptosis following ginsenoside Rk1 treatment. Exposure led to a substantial increase in bright blue nuclear staining, confirming the induction of cell death. (b) Flow cytometry scatter plots. (c) Apoptotic rates in HeLa cells demonstrated clear elevation with rising ginsenoside Rk1 concentrations relative to untreated controls.

Compared with the blank control group, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Additional analysis using Annexin V/PI dual staining via flow cytometry revealed higher apoptotic fractions in all ginsenoside Rk1-treated samples compared to controls (**Figures 2b and 2c**). Apoptotic percentages rose dose-dependently from  $4.28 \pm 0.47\%$  in untreated cells to  $12.39 \pm 0.99\%$  at the highest dose examined ( $p < 0.001$ ). Collectively, these outcomes highlight that ginsenoside Rk1 suppresses HeLa cell growth predominantly through apoptotic mechanisms.

#### Ginsenoside Rk1 affects immune protein expression

The influence of ginsenoside Rk1 on apoptosis-related proteins was examined by western blotting, focusing on caspase 3, caspase 6, PARP, and LC3B in HeLa cells. Treated groups displayed elevated protein abundance for caspase 3, caspase 6, PARP, and LC3B compared with untreated controls (**Figures 3a and 3b**). Significant increases in caspase 3 and caspase 6 were recorded ( $p < 0.05$ ), while PARP and LC3B showed dose-dependent upregulation at 20  $\mu\text{M}$  and 30  $\mu\text{M}$  concentrations ( $p < 0.01$ ). These changes imply that ginsenoside Rk1 stimulates apoptosis by engaging the caspase 3-, PARP-, and caspase 6-dependent cascades. The concurrent rise in LC3B further indicates stimulation of autophagy pathways.



**Figure 3.** Protein expression levels in HeLa cells after ginsenoside Rk1 treatment. (a) and (b) Significant upregulation of caspase 3, caspase 6, PARP, and LC3B proteins occurs in response to ginsenoside Rk1

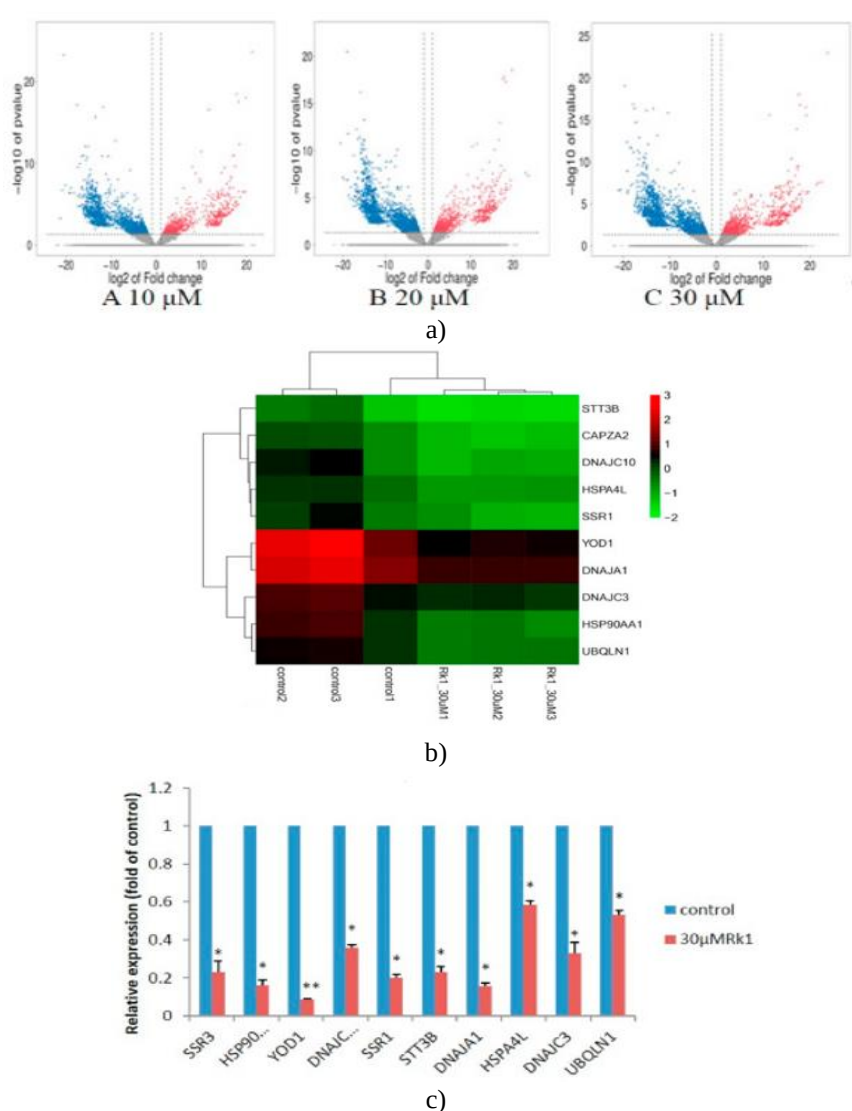
compared with untreated controls. (c) and (d) Marked downregulation of YOD1, HSPA4L, DNAJC3, and HSP90AA1 proteins is evident following ginsenoside Rk1 exposure. YOD1 demonstrated the greatest sensitivity, with expression reduced by approximately five-fold relative to controls. Compared to the blank control group, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

#### *Rk1 treatment alters gene expression in HeLa cells*

To assess the transcriptomic consequences of ginsenoside Rk1 on human HeLa cells, four experimental cohorts were prepared, including untreated controls and cells receiving the compound. RNA from these groups was processed into cDNA libraries for sequencing, generating robust transcriptome datasets. Quality assessment of cleaned reads showed an average Q30 score of 98.3% and an effective read proportion of 97.52%, supporting the high reliability of the sequencing output.

#### *Volcano map analysis of DEGs*

Volcano plots provided an overview of the distribution and magnitude of differentially expressed genes (DEGs). Responsiveness to Rk1 was gauged by comparing expression profiles between treated and control samples. With thresholds set at  $FC \geq 2$  and  $FDR < 0.05$ , 4,113 DEGs were detected in the 10  $\mu\text{M}$  ginsenoside Rk1 group versus control (**Figure 4a**), comprising 187 upregulated and 3,926 downregulated genes. The 20  $\mu\text{M}$  treatment yielded 5,016 DEGs (534 upregulated, 4,482 downregulated), while the 30  $\mu\text{M}$  condition produced 5,438 DEGs (766 upregulated and 4,582 downregulated).

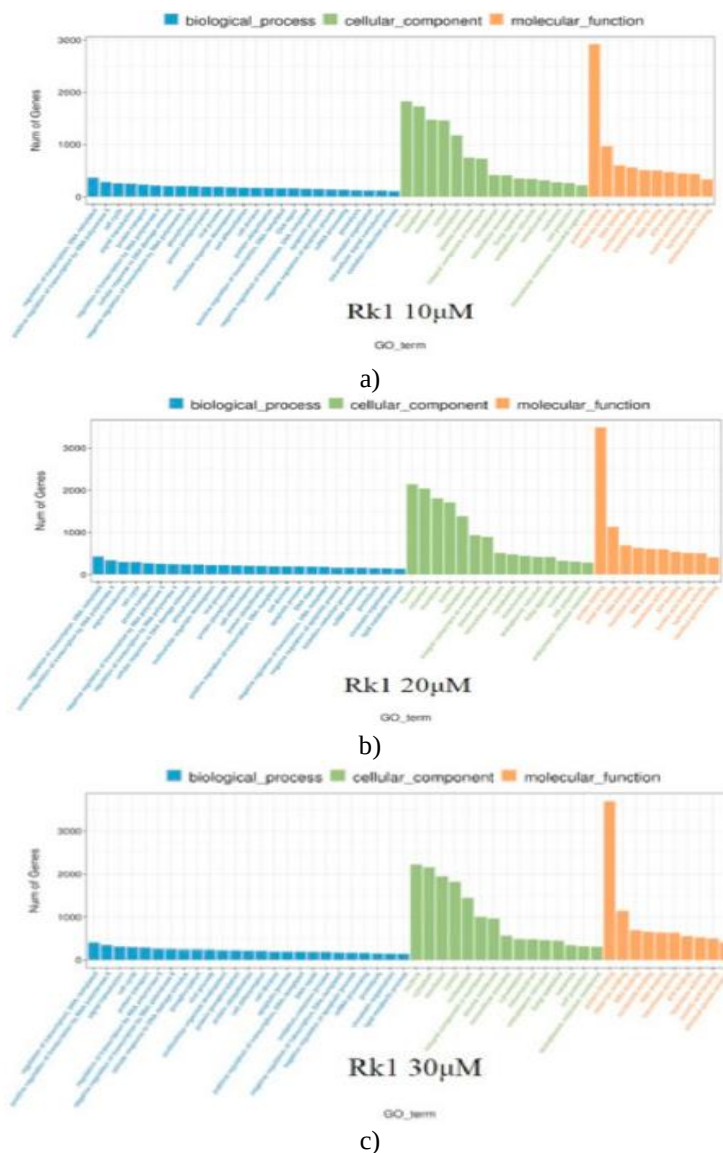


**Figure 4.** Differential gene expression after ginsenoside Rk1 treatment. (a) MA plot depicting gene expression changes in HeLa cells exposed to ginsenoside Rk1. Differential expression was calculated

between treated and untreated samples. The quantity of DEGs rose substantially as ginsenoside Rk1 concentration increased. (b) Heatmap of selected DEGs, with color depth indicating the extent of expression alteration. (c) Validation by RT-qPCR of ten DEGs linked to the protein processing in the endoplasmic reticulum pathway (SSR3, SSR1, YOD1, HSPA4L, HSP90AA1, STTSB, DNAJC3, UBQLN1, DNAJA1, and DNAJC10). YOD1 exhibited particularly strong inhibition by ginsenoside Rk1. Its expression pattern remained consistent across RNA-seq, western blotting, and RT-qPCR analyses.

#### Gene ontology enrichment analysis of differentially expressed genes following ginsenoside Rk1 treatment

Each set of differentially expressed genes (DEGs) was categorized into three primary GO domains: molecular function, cellular component, and biological process. Among the top 50 most enriched terms in each domain, molecular functions were dominated by activities involving protein binding, metal ion binding, and DNA binding (**Figure 5**). The DEGs were primarily associated with the membrane, cytoplasm, and nucleus at the cellular component level. At the biological process level, they were notably linked to the cell cycle, DNA templating, positive regulation of RNA polymerase II-mediated transcription, and general transcriptional control. These patterns corroborate earlier findings showing that HeLa cells, unlike normal cervical tissue, are highly enriched in pathways connected to intracellular transport, cytoskeletal elements, protein folding, cell cycle control, and programmed cell death.



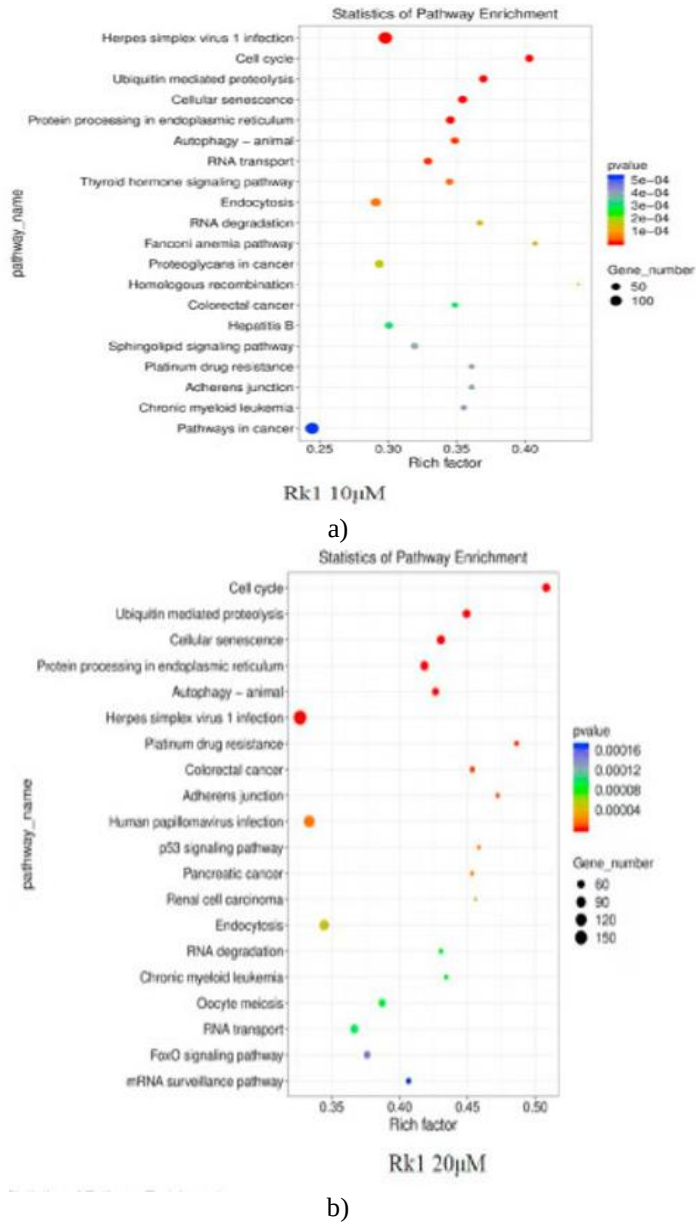
**Figure 5.** GO enrichment map of DEGs associated with ginsenoside Rk1 treatment. Each group of DEGs was annotated into three classifications: molecular functions, cellular components, and biological processes. In

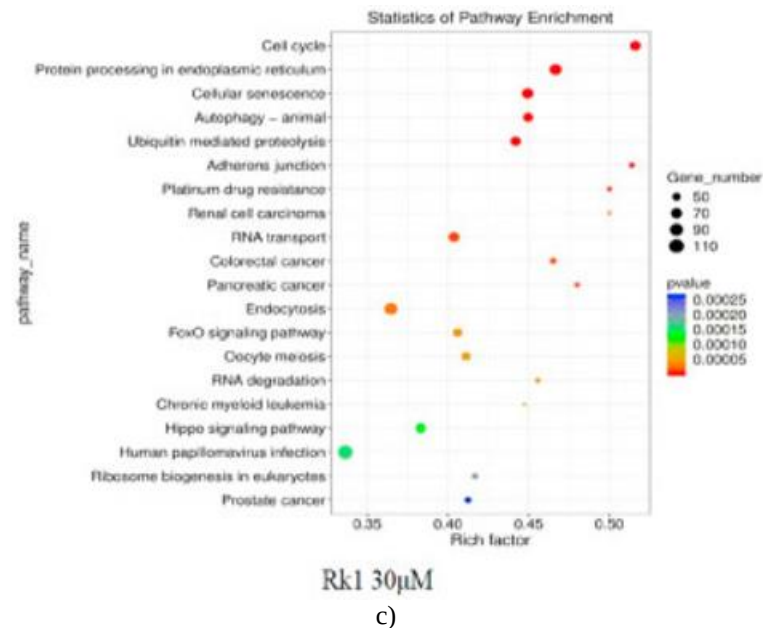
descending order from largest to smallest, the top 25, top 15, and top 10 GO terms were selected, respectively, for visualization.

*KEGG pathway enrichment analysis of DEGs induced by ginsenoside Rk1 treatment*

In biological systems, coordinated gene activity underlies most cellular functions. Analyzing these activities at the pathway level offers important insights into gene roles. The KEGG database is the main resource for such systematic pathway investigations.

A large proportion of cell cycle regulators (such as CDC27, GSK3B, TGFB2, CDC6, CDC23, and CDC7) were strongly enriched in pathways governing cell proliferation (**Figure 6**). Genes participating in cellular senescence signaling, including CCNA2, CDKN2B, ATM, CHEK1, FOXO1, and ITPR1, were also significantly overrepresented within the cell cycle DEGs responsive to Rk1. Across the three concentrations tested, the protein processing in the endoplasmic reticulum pathway stood out as one of the most highly enriched. In this pathway, several prominent genes—including YOD1, HSPA4L, DNAJC3, and HSP90AA1—were downregulated. These genes are essential for proper protein folding, maturation, and transport in the endoplasmic reticulum and contribute to cellular resistance to apoptosis.





**Figure 6.** KEGG pathway analysis of DEGs enriched in response to ginsenoside Rk1 treatment. The horizontal axis represents the enrichment factor and the vertical axis represents the enriched KEGG pathway. The dot size indicates the number of DEGs enriched in a KEGG pathway. Dot colors represent different p-values associated with the DEGs. The enrichment factor indicates the number of DEGs in a KEGG pathway/the total number of genes belonging to this KEGG pathway. The larger the enrichment factor, the higher the enrichment degree for that KEGG pathway.

#### *Hierarchical clustering of DEGs in the endoplasmic reticulum protein processing pathway*

DEGs participating in protein processing within the endoplasmic reticulum pathway were analyzed, and ten key genes were presented in a hierarchical clustering diagram (**Figure 4b**). All examined DEGs (SSR3, SSR1, HSPA4L, HSP90AA1, STTSB, YOD1, DNAJC3, UBQLN1, DNAJA1, and DNAJC10) exhibited decreased expression.

#### *RT-qPCR Validation of Genes Associated with Ginsenoside Rk1 Treatment*

RT-qPCR assays were used to confirm the RNA-seq findings. Ten selected DEGs from the endoplasmic reticulum protein processing pathway (SSR3, SSR1, YOD1, HSPA4L, HSP90AA1, STTSB, DNAJC3, UBQLN1, DNAJA1, and DNAJC10) all showed statistically significant downregulation ( $p < 0.05$ ) (**Figure 4c**). YOD1 expression was particularly suppressed ( $p < 0.01$ ) after ginsenoside Rk1 exposure, matching the direction of change seen in the standardized hierarchical clustering results (**Figure 4b**). Together, these data support the idea that ginsenoside Rk1 modulates genes responsible for protein production and handling in the endoplasmic reticulum.

#### *Western blot analysis of endoplasmic reticulum pathway proteins following ginsenoside Rk1 treatment*

Western blotting was conducted to evaluate protein abundance of key endoplasmic reticulum pathway components. The results provided insight into how ginsenoside Rk1 affects protein maturation in the endoplasmic reticulum and the suppression of apoptosis in HeLa cells. Levels of YOD1, HSPA4L, DNAJC3, and HSP90AA1 were substantially reduced in all treatment groups compared to untreated controls (**Figures 3c and 3d**). Protein expression decreased in a concentration-dependent manner as ginsenoside Rk1 levels rose, highlighting its dose-dependent cytotoxicity toward HeLa cells. Elevated concentrations led to stronger inhibition of protein synthesis and processing as well as suppression of anti-apoptotic factors. The most pronounced effect was observed on YOD1 ( $p < 0.001$ ). While HSPA4L, DNAJC3, and HSP90AA1 each showed roughly three-fold reduction, YOD1 levels declined almost five-fold ( $p < 0.01$ ). These findings indicate that the cytotoxicity of ginsenoside Rk1 in HeLa cells is largely attributable to impaired endoplasmic reticulum protein production and disruption of anti-apoptotic defenses.

Cervical cancer ranks as the fourth most common malignancy among women worldwide [11] and represents the leading malignant tumor of the female reproductive tract in China. While early-stage cervical cancer has a favorable prognosis, patient outcomes deteriorate markedly once metastasis or recurrence develops [12]. Previous investigations have shown that 20(S)-ginsenoside Rh2 induces protective autophagy and enhances cervical cancer cell death by inhibiting the AMPK/mTOR signaling axis [13]. Similarly, ginsenoside Rg5 has been found to suppress NF $\kappa$ B activation by blocking its upstream regulator, transforming growth factor  $\beta$ -activated kinase 1, in TNF- $\alpha$ -stimulated HeLa and A549 cells [14]. Ginsenoside Rk1, a principal bioactive constituent of ginseng, was observed in the present study to inhibit HeLa cell proliferation in a dose- and time-dependent fashion. Hoechst 33258 staining further revealed a progressive rise in the proportion of apoptotic cells as ginsenoside Rk1 concentration increased.

Among the caspase family members, caspase-3 serves as a critical executioner of apoptosis, mediating cytoskeletal breakdown, nuclear fragmentation, and other hallmark apoptotic alterations [15]. PARP functions as a key substrate of activated caspase-3 during apoptotic execution [16], whereas caspase-6 acts as a downstream effector in this proteolytic cascade [17]. Upon receiving apoptotic stimuli, these proteins undergo sequential activation, leading to the cleavage of target substrates and the initiation of programmed cell death. LC3 constitutes a central component of the autophagic machinery, facilitating the transport of autophagy-related signals and factors [18]. Western blot analysis comparing untreated control cells with ginsenoside Rk1-treated cells demonstrated markedly elevated protein levels of PARP, caspase-6, caspase-3, and LC3B in the treated group. These changes indicate that ginsenoside Rk1 stimulates apoptotic signaling through the caspase-3/PARP/caspase-6 pathway. In addition, the substantial upregulation of the autophagy marker LC3B suggests that ginsenoside Rk1 activates autophagy in HeLa cells via autophagic signaling routes.

The suppression of cervical tumor cell growth is a multifaceted process involving numerous regulatory steps and intricate biological networks, frequently accompanied by multiple genetic mutations and dysregulated gene expression [19]. In the current investigation, RNA-seq was employed to identify DEGs in HeLa cells, followed by comprehensive functional enrichment analyses. The endoplasmic reticulum (ER) not only serves as the primary site for protein folding [20, 21] but also functions as a major intracellular calcium reservoir [22] and hosts molecular chaperones essential for protein folding and quality control [23]. These components play pivotal roles in modulating cellular sensitivity to ER stress and apoptosis. The ER quality control system relies on various molecular chaperones that assist in proper protein folding and trafficking. When folding fails, chaperones attempt refolding; irreparably misfolded proteins are subsequently directed toward proteasomal degradation or autophagy [24]. Excessive accumulation of misfolded proteins triggers cellular toxicity and activates apoptotic pathways, ultimately resulting in cell death.

In this study, several key “hotspot” genes within the ER protein processing pathway were significantly downregulated in HeLa cells following ginsenoside Rk1 exposure, including YOD1, HSPA4L, DNAJC3, and HSP90AA1. YOD1, a deubiquitinating enzyme, has previously been reported to be overexpressed in uterine leiomyoma cells [25]. HSPA4L has emerged as a novel oncogene in pheochromocytoma [26], DNAJC3 shows elevated expression in breast cancer [27], and HSP90AA1 represents a prominent pan-cancer target gene involved in ER protein maturation and trafficking as well as in conferring resistance to apoptosis [28]. Although ginsenoside Rk1 appears to modulate ER protein folding through these targets in HeLa cells, it remains to be determined whether these genes directly contribute to the initiation and progression of cervical cancer in clinical settings.

Importantly, protein expression levels of these targets declined progressively with increasing ginsenoside Rk1 concentrations, confirming a clear dose-dependent response in HeLa cells. Higher doses produced more pronounced suppression of protein synthesis and processing as well as greater inhibition of anti-apoptotic gene expression. In particular, YOD1 expression was strongly repressed by ginsenoside Rk1 ( $p < 0.001$ ), exhibiting nearly five-fold downregulation relative to untreated controls. Specifically, normalized YOD1 expression decreased from approximately 0.5 in untreated cells to approximately 0.1 at the highest ginsenoside Rk1 concentration. These observations indicate that the cytotoxic activity of ginsenoside Rk1 against HeLa cells arises primarily from the inhibition of ER protein synthesis and the downregulation of anti-apoptotic factors, consistent with findings from prior research.

## Conclusion

RNA-seq data further revealed that YOD1 expression was not only markedly reduced following ginsenoside Rk1 treatment but also displayed stable expression patterns. Consequently, YOD1 emerges as a promising molecular target for the potential clinical management of cervical cancer.

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**Conflict of Interest:** None

**Financial Support:** None

**Ethics Statement:** None

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