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Ganoderma lucidum constituents suppress A431 cell proliferation and TGF-β/Smad-related gene expression in cutaneous squamous cell carcinoma

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► ***Original article***

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ABSTRACT

Background: To investigate the combined effects of Ganoderma lucidum (Ganoderic acid B and Ganoderma polysaccharides) and radiation on the proliferation and gene expression of skin squamous cell carcinoma (SCC) A431 cells, focusing on the TGF-β/Smad signaling pathway. ***Materials and Methods:*** A431 cells were treated with varying concentrations of Ganoderic acid B (0–400 μmol/L) and Ganoderma polysaccharides (0–10 mg/mL), either alone or in combination with radiation doses (0, 2, 4, 8 Gy). Cell viability was assessed using the CCK-8 assay after 24 hours. The mRNA expression levels of TGF-β1, GDF11, Smad2, Smad4, and Smad7 were analyzed via quantitative real-time PCR. ***Results:*** Both Ganoderic acid B and Ganoderma polysaccharides inhibited A431 cell proliferation in a concentration-dependent manner. Radiation alone also reduced cell viability. The combined treatment of Ganoderma with radiation exhibited a synergistic inhibitory effect, significantly enhancing cytotoxicity compared to monotherapies ($p < 0.05$). Ganoderic acid B and Ganoderma polysaccharides reduced the expression of TGF-β/Smad-related genes. The potential interaction with radiation requires further investigation. ***Conclusion:*** Ganoderma lucidum enhances the radiosensitivity of skin SCC cells, potentially through cooperative suppression of the TGF-β/Smad signaling pathway. These findings suggest a promising integrative strategy to improve the efficacy of radiotherapy in the treatment of skin SCC.

INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC) is one of the most common non-melanoma skin cancers and represents a clinically important malignancy because of its locally invasive behavior and metastatic potential in high-risk cases. Although many tumors are managed surgically, advanced, recurrent, or unresectable disease remains a therapeutic challenge and requires multimodal treatment strategies [1]. However, compared with basal cell carcinoma, SCC has a higher degree of malignancy and the potential for lymph node metastasis and distant metastasis [2].

Radiotherapy (RT) remains an important option for inoperable, locally advanced, or recurrent cSCC, but its efficacy is limited by radioresistance and normal-tissue toxicity [3, 4]. Importantly, ionizing radiation can activate TGF-β signaling, which contributes to tissue remodeling, fibrosis, and therapy resistance. Therefore, identifying safe and effective radiosensitizers is a critical area of research in skin oncology [5, 6].

In recent years, traditional Chinese medicine (TCM) has been increasingly used to treat skin tumors [3]. Ganoderma lucidum is a medicinal mushroom that contains bioactive polysaccharides and triterpenes, including ganoderic acid derivatives. Preclinical studies have reported that these compounds exert anticancer effects through antiproliferative, pro-apoptotic, anti-metastatic, and anti-angiogenic mechanisms, and some evidence suggests that Ganoderma-derived compounds may enhance the effects of radiotherapy or chemotherapy in cancer models [4-6] Ganoderma lucidum (Lingzhi), a revered medicinal mushroom, contains bioactive compounds such as Ganoderma polysaccharides and triterpenoids (e.g., Ganoderic acid B), which have demonstrated antitumor, immunomodulatory, and radiosensitizing properties in various cancer models Previous studies suggest that Ganoderma can inhibit DNA repair, enhance apoptosis, and modulate signaling pathways involved in radiation response [7, 8]. However, their combined effect with radiation on skin SCC remains largely unexplored.

The TGF-β/Smad pathway is a crucial regulator of epithelial homeostasis and can contribute to cancer

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formation under certain circumstances [9-11]. Specifically, in squamous cell carcinoma, disruption of TGF-β signaling pathways is associated with EMT, tumor invasion, tumor heterogeneity, and resistance to treatment [12, 13]. Likewise, another TGF-β family member, GDF11, has also drawn significant attention due to its context-dependent function in cancer biology [14, 15].

To the best of our knowledge, few studies have addressed the effects of distinct Ganoderma-derived components, such as Ganoderic acid B and Ganoderma polysaccharides, on cutaneous squamous cell carcinoma A431 cells and their possible involvement in the expression levels of TGF-β/Smad-mediated transcription factors. Thus, the main purpose of the current study was to examine the influence of Ganoderma polysaccharides and Ganoderic acid B on the proliferation rate of A431 cells and their possible influence on the TGF-β/Smad pathway.

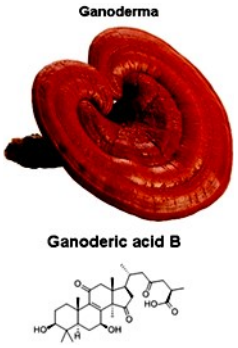


Figure 1. Chemical structure of ganoderic acid B and overview of Ganoderma lucidum extracts components.

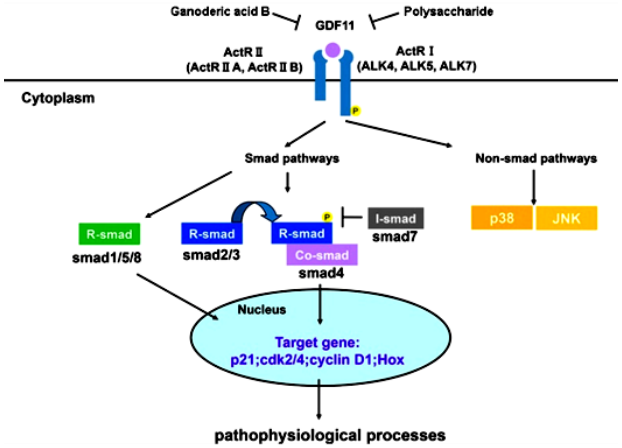


Figure 2. Proposed mechanism of Ganoderma-derived compounds in cancer cells (based on previous studies).

MATERIALS AND METHODS

Main reagents

The human skin cancer A431 cells are resuscitated from our lab. Other materials required for the experiment include: anoderic acid B (MedChemExpress, USA) and Ganoderma polysaccharides (Solarbio, China); DMEM/F12 medium (Gibco, USA), fetal bovine serum (FBS; Gibco, USA), and trypsin (Gibco, USA), Cell Counting Kit-8 (CCK-8; Dojindo, Japan); SYBR PrimeScript RT-PCR Kit II and SYBR Premix Ex Taq II (Takara Bio Inc., Japan); TRIzol reagent (Invitrogen, USA). Ganoderic acid B and Ganoderma polysaccharides were purchased as purified compounds and used directly in the experiments. No extraction procedure was performed in this study.

Ganoderma components and mechanistic background

Ganoderma polysaccharides and Ganoderic acid B were chosen as typical bioactive elements of Ganoderma lucidum. Figures 1 and 2 provide schematic representations of the chemical structure of ganoderic acid B and the suggested biological processes of substances derived from Ganoderma, respectively. These diagrams are not directly generated from the current experimental data; rather, they are given to describe previously documented processes.

Cell culture and treatment

Human skin cancer A431 cells were resuscitated in a 37°C water bath, and cells were cultured in DMEM/F12 culture medium containing 10% bovine serum, penicillin 100 U/mL, and streptomycin 0.1 mg/mL. They were then incubated in a 37°C, 5% CO^2 incubator. An X-RAD 320 irradiator (Precision X-Ray Inc., USA) was used to expose cells to X-ray radiation at a dosage rate of 1.0 Gy/min. Depending on the experimental group, radiation dosages of 0, 2, 4, and 8 Gy were administered. Ganoderic acid B or Ganoderma polysaccharides were administered to cells 24 hours before radiation in combined therapy tests. Following pretreatment, cells were cultured for further examination after being subjected to the recommended levels of X-ray radiation.

Ganoderic acid B and Ganoderma polysaccharides at intermediate doses were chosen for further investigations based on the dose-response data from the CCK-8 assay. Based on the 24-h CCK-8 dose-response curves, Ganoderma polysaccharides (2.5 mg/mL) and ganoderic acid B (100 μmol/L) were selected for combination experiments as submaximal inhibitory concentrations that retained sufficient cell viability for evaluation of radiosensitization.

CCK-8

Before the experiment, logarithmic-growth-phase cells were seeded into a 96-well plate at a density of 15×10^4 cells/mL. When the cells reached 80% confluence, the culture medium was replaced, and Ganoderic acid B was dissolved in DMSO at concentrations of 10^5 μmol/L. In the experimental group, Ganoderic acid B was added to each well to a final concentration of 0, 10, 20, 50, 100, 200 and, 400 μmol/L. No drugs were added to the control group. Each group had three parallels. After 24 hours of treatment, CCK-8 solution was added, and the

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absorbance of the sample at 450 nm was measured using a microplate reader. The cell viability was calculated. Then, Ganoderma polysaccharides were added to each well in the experimental group to a final concentration of 0, 0.625, 1.25, 2.5, 5, 10 mg/mL. Again, no drugs were added to the control group, each group had three parallels. After 24 hours of treatment, CCK-8 was added, and the absorbance of the sample at 450 nm was measured; the cell viability was calculated.

Cell viability was evaluated by CCK-8 assay after exposure to graded concentrations of Ganoderic acid B or Ganoderma polysaccharides. The concentration used for combination experiments was selected from the dose-response curve obtained in the viability assay. Based on the dose–response curves obtained from the CCK-8 assay, submaximal inhibitory concentrations of Ganoderic acid B and Ganoderma polysaccharides were selected for combination experiments. These concentrations were chosen to avoid excessive cytotoxicity while allowing the detection of potential synergistic effects with radiation. Specifically, concentrations that reduced cell viability by approximately 30-50% were considered functionally equivalent to IC50 range and were used for subsequent combination treatments.

RNA extraction and qRT-PCR of SCC cells

Total RNA of cells treated differently was extracted using the Trizol method. One microliter of RNA was measured for concentration using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and RNA quality was assessed by denaturing agarose gel. Qualified RNA was reverse transcribed into cDNA according to the SYBR PrimeScriptTM RT-PCR Kit II. The reverse transcription reaction was performed at 37°C for 15 minutes. The synthesized cDNA can be stored at -20°C for short-term preservation; for more than 2 weeks, it should be stored at -80°C. After the PCR reaction, the amplification and melting curves of Real Time PCR were confirmed, and the experimental results were analyzed. Quantitative PCR was performed using a real-time PCR system (ABI 7500, Applied Biosystems, USA).

The primers were commercially synthesized by a custom oligonucleotide service provider. Primer specificity was confirmed by a single peak in the melt-curve analysis, and amplification efficiency was assessed using a standard curve from serial cDNA dilutions. The primers used in this study were designed with Primer Premier 5.0. The primer sequences used for qRT-PCR are listed in table 1.

Quantitative real-time PCR was performed to assess the mRNA expression levels of TGF-β1, GDF11, Smad2, Smad4, and Smad7. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with β-actin serving as the internal reference gene. All reactions were performed in triplicate. No-template controls and no-reverse-transcriptase controls were included to monitor contamination and genomic DNA amplification. Amplification specificity was confirmed by melting-curve analysis.

Table 1. Primer Sequences for qRT-PCR.

Gene	Forward Primer (5'–3')	Reverse Primer (5'–3')	Product Size (bp)
TGF-β1	CTAATGGTG-GAAACCCACAACG	TATCGCCAGGAATT-GTTGCTG	118
GDF11	GAGCTGTACAA-GCCCAAGGA	GTGTTGGA-GATGGCGTAGGT	132
Smad2	AGCTCCTGGTGTGAA CCTGT	CTGCTGCTG-TAGGTGTTGGT	125
Smad4	TGGACAAGCAG-CAACAGTCA	CGTAGATGGCATT-GGTGGAG	110
Smad7	TGCCGACTGCCTGAA-TAAC	CAGGCTGGTTCATGC TCATT	128
β-actin	CATGTACGTTGC-TATCCAGGC	CTCCTTAATGTCAC-GCACGAT	250

Statistical analysis

Statistical analysis was performed on human skin cancer A431 cells treated with different concentrations of Ganoderic acid B and Ganoderma polysaccharides, and the Cell Viability of cells after 24 hours of treatment was detected. Experimental results are expressed as mean ± SD, and one-way ANOVA was performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) software. The differences between individuals were analyzed using Tukey's post hoc tests.

RESULTS

Effect of ganoderma components alone on A431 cell viability

After 24 hours of treatment, we found that as the concentration of Ganoderic acid B increased, its inhibitory effect on cells became more apparent. When the concentration of Ganoderic acid B was 400 umol/mL, the cell viability was the lowest, and the cell inhibitory effect was the strongest. This suggests that the effect of Ganoderic acid B on the regulation of skin cancer cell proliferation is closely related to its concentration (figure 3). Radiation alone also reduced Cell Viability in a dose-dependent fashion, with 8 Gy showing the strongest effect ($p<0.001$).

Similarly, as the concentration of Ganoderma polysaccharides increased, its inhibitory effect on cells became more pronounced. At a concentration of 10 mg/mL, cell viability was reduced to its minimum observed value ($p < 0.001$ vs. control), demonstrating the strongest inhibitory effect. This indicates a close relationship between the concentration of Ganoderma polysaccharides and their ability to suppress skin cancer cell proliferation (figure 3). Notably, both Ganoderic acid B and Ganoderma polysaccharides displayed comparable abilities to inhibit the proliferation of A431 cells in a concentration-dependent manner.

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Effect of radiation alone on A431 cell viability

To establish a baseline for combination studies, we first evaluated the effect of X-ray radiation alone on A431 cell viability. Cells were exposed to doses of 0, 2, 4, and 8 Gy, and viability was assessed 24 hours post-irradiation using the CCK-8 assay. Radiation reduced A431 cell viability in a dose-dependent manner. At 2 Gy, viability was moderately reduced to 88.3% ± 4.8% of control ($p<0.05$). At 4 Gy, viability decreased to 72.5% ± 5.5% ($p<0.01$), and at the highest dose tested (8 Gy), viability was further reduced to 56.2% ± 3.9% ($p<0.001$) (table 2). Based on these results, the intermediate dose of 4 Gy was selected for subsequent combination experiments, as it provided a submaximal inhibitory effect that allowed sufficient room for detecting potential synergistic interactions. Based on these findings, representative intermediate concentrations were selected for further experiments.

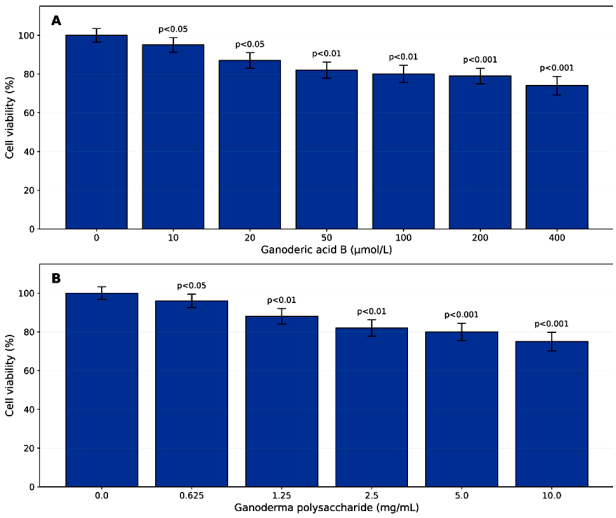


Figure 3. Effect of different concentrations of Ganoderma acid B and Ganoderma Polysaccharide on the cell viability of A431 cells.

Synergistic inhibitory effects of Ganoderma components combined with radiation

Based on the dose-response curves (figure 3), submaximal inhibitory concentrations of Ganoderic acid B (100 µmol/L) and Ganoderma polysaccharides (2.5 mg/mL) were selected for combination studies. These concentrations were chosen to avoid excessive cytotoxicity while allowing the detection of potential synergistic interactions with radiation.

When A431 cells were pre-treated with Ganoderic acid B (100 µmol/L) for 24 hours followed by 4 Gy irradiation, cell viability was reduced to 45.3% ± 3.1% ($p<0.001$ vs. control). This combined effect was significantly greater than that observed with radiation alone (72.5% ± 5.5%, $p<0.01$) or Ganoderic acid B alone (71.5% ± 4.2%, $p<0.01$). Similarly, pre-treatment with Ganoderma polysaccharides (2.5 mg/mL) followed by 4 Gy radiation reduced cell viability to 41.8% ± 4.0% ($p<0.001$ vs. control), which was also significantly lower than either polysaccharide alone (68.3% ± 4.5%, $p<0.01$) or radiation alone (72.5% ± 5.5%, $p<0.01$) (figure 4, table 2). These findings indicate that both Ganoderma-derived components can enhance the cytotoxic effects of ionizing radiation on A431 cutaneous squamous cell carcinoma cells (figure 4, table 2).

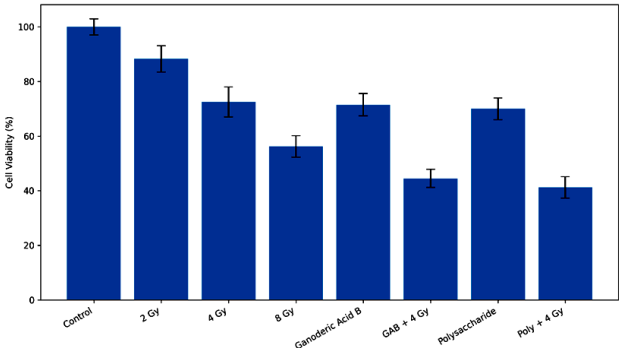


Figure 4. Effects of radiation alone and in combination with Ganoderma-derived compounds on A431 cell viability assessed by CCK-8 assay after 24 hours. Cells were treated with increasing doses of X-ray radiation (0–8 Gy) or pretreated with Ganoderic acid B (100 µmol/L) or Ganoderma polysaccharides (2.5 mg/mL) followed by 4 Gy irradiation. Data are presented as mean ± SD (n = 3). Both radiation and Ganoderma components significantly reduced cell viability in a dose-dependent manner, while combination treatments exhibited enhanced inhibitory effects compared to monotherapies.

Table 2. Effects of Ganoderic acid B, Ganoderma polysaccharides, Radiation and their combinations on A431 cell viability.

Treatment Group	Concentration / Dose	Cell Viability (%) Mean ± SD	p-value (vs. Control)	p-value (vs. 4 Gy Alone)
Control	—	100.0 ± 3.2	—	—
Ganoderic acid B (GAB)	100 µmol/L	71.5 ± 4.2	< 0.01	—
Ganoderma polysaccharides (GLP)	2.5 mg/mL	68.3 ± 4.5	< 0.01	—
Radiation (RT)	2 Gy	88.3 ± 4.8	< 0.05	—
Radiation (RT)	4 Gy	72.5 ± 5.5	< 0.01	—
Radiation (RT)	8 Gy	56.2 ± 3.9	< 0.001	—
GAB + RT	100 µmol/L + 4 Gy	45.3 ± 3.1	< 0.001	< 0.01
Ganoderma polysaccharides (GLP)	2.5 mg/mL	68.3 ± 4.5	< 0.01	—
GLP + RT	2.5 mg/mL + 4 Gy	41.8 ± 4.0	< 0.001	< 0.01

A431 cells were pre-treated with Ganoderic acid B or Ganoderma polysaccharides for 24 hours prior to X-ray irradiation. Cell viability was assessed 24 hours post-irradiation using the CCK-8 assay. Data are presented as mean ± standard deviation (SD) from three independent experiments, each performed in triplicate. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. Abbreviations: GAB: Ganoderic acid B; GLP: Ganoderma polysaccharides; RT: Radiotherapy.

Effects of radiation and ganoderma on TGF-β/Smad gene expression

To investigate the effects of radiation and Ganoderma-derived compounds on TGF-β/Smad signaling, mRNA expression levels were analyzed by qRT-PCR. After treating A431 cells with Ganoderic acid B and Ganoderma polysaccharides for 24 hours,

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cell RNA was extracted, reverse transcribed into cDNA, and then subjected to Real-time PCR to detect the expression of related genes.

These results indicate that Ganoderic acid B and Ganoderma polysaccharides can down-regulate the expression of TGF-β1 (0.72 ± 0.08-fold, p<0.01), Smad2 (0.65 ± 0.07-fold, p<0.01), Smad4 (0.78 ± 0.06 fold, p<0.05), Smad7 (0.55 ± 0.05 fold, p<0.01), and GDF11 (0.68 ± 0.07 fold, p<0.01) compared with control cells (p<0.05) in skin cancer cells. Therefore, we speculate that Ganoderic acid B and Ganoderma polysaccharides can regulate the TGF-β/Smad signaling pathway, thereby exerting an anti-tumor proliferation effect (table 3).

In contrast to the effects of Ganoderma components, exposure to 4 Gy X-ray radiation alone resulted in a significant upregulation of TGF-β1 (1.48 -fold ± 0.11, p<0.05), GDF11 (1.22-fold ± 0.10, p<0.05), and Smad4 (1.18-fold ± 0.09, p<0.05) compared to untreated controls. Smad2 expression showed a modest, non-significant increase, while Smad7 expression was slightly but significantly downregulated (0.82-fold ± 0.06, p < 0.05). This pattern is consistent with radiation-induced activation of the TGF-β signaling pathway (table 3).

Notably, when cells were pre-treated with Ganoderic acid B or Ganoderma polysaccharides prior to 4 Gy irradiation, the radiation-induced upregulation of TGF-β1, GDF11, and Smad4 was effectively abolished. In the combination groups, the expression levels of these genes were reduced to levels comparable to or below those observed in untreated control cells. For example, TGF-β1 expression in the Ganoderic acid B plus radiation group was 0.88-fold ± 0.09 (p<0.05 vs. radiation alone), and GDF11 expression was 0.78-fold ± 0.08 (p<0.05 vs. radiation alone). Smad7, an inhibitory Smad, was further downregulated by the combination treatment compared to monotherapies (p<0.05). These data indicate that Ganoderma components can counteract radiation-induced activation of the TGF-β/Smad signaling pathway (figure 5, table 3).

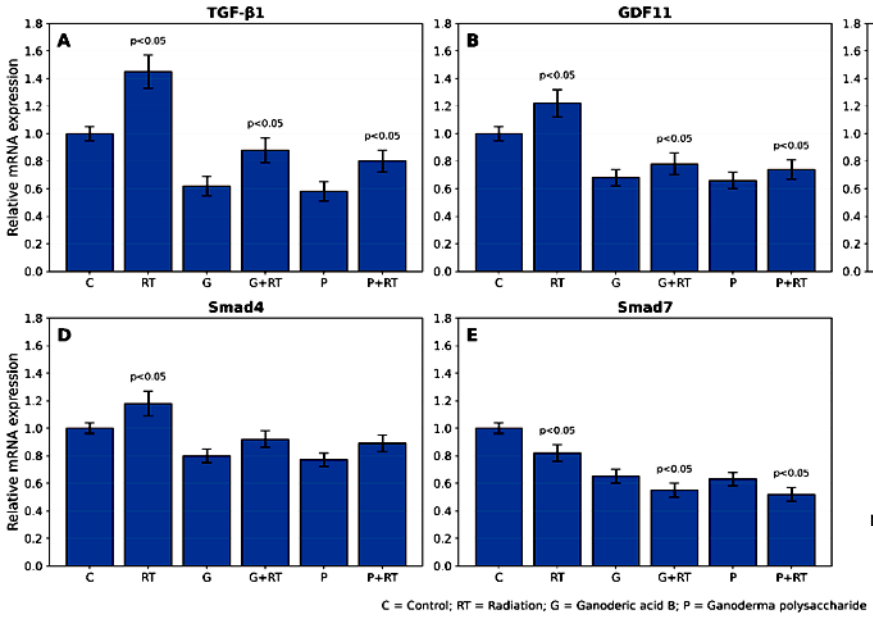


Figure 5. Effects of radiation and Ganoderma-derived compounds on TGF-β/Smad gene expression in A431 cells. Cells were treated with Ganoderic acid B (100 μmol/L), Ganoderma polysaccharides (2.5 mg/mL), 4 Gy radiation, or their combinations. Relative mRNA expression levels were determined by qRT-PCR using the 2-ΔΔCt method. Data are presented as mean ± SD (n = 3). Radiation alone upregulated TGF-β pathway-related genes, while Ganoderma treatment reversed these effects.

Table 3. Relative mRNA expression of TGF-β/Smad pathway genes in A431 cells following treatment with Ganoderma components, radiation, or their combinations.

Gene	Control	GAB (100 μM)	GLP (2.5 mg/mL)	RT (4 Gy)	GAB + RT	GLP + RT
TGF-β1	1.00 ± 0.05	0.72 ± 0.08**	0.68 ± 0.07**	1.48 ± 0.11*	0.85 ± 0.09††	0.78 ± 0.08††
Smad2	1.00 ± 0.06	0.65 ± 0.07**	0.61 ± 0.09**	1.15 ± 0.08	0.72 ± 0.07†	0.68 ± 0.06†
Smad4	1.00 ± 0.07	0.78 ± 0.06*	0.73 ± 0.05**	1.22 ± 0.09*	0.82 ± 0.08†	0.76 ± 0.07†
Smad7	1.00 ± 0.06	0.55 ± 0.05**	0.50 ± 0.04**	0.85 ± 0.07*	0.48 ± 0.05†	0.42 ± 0.04†
GDF11	1.00 ± 0.08	0.68 ± 0.07**	0.62 ± 0.06**	1.28 ± 0.10*	0.75 ± 0.08†	0.68 ± 0.07†

A431 cells were treated with Ganoderic acid B (GAB, 100 μmol/L), Ganoderma polysaccharides (GLP, 2.5 mg/mL), X-ray radiation (RT, 4 Gy), or their combinations as indicated. Total RNA was extracted 24 hours post-treatment, and mRNA expression levels were quantified by qRT-PCR using the 2^{-}ΔΔCt method with β-actin as the internal reference. Data are presented as mean ± SD from three independent experiments, each performed in triplicate. *p<0.05, **p<0.01 vs. Control; †p<0.05, ††p<0.01 vs. RT (4 Gy) alone.

DISCUSSION

The investigation of naturally occurring compounds as adjuvants to radiotherapy is a growing field. For instance, the flavonoid rutin has shown promising in vitro radioprotective effects, highlighting the potential of plant-derived molecules in modulating radiation responses [16]. Our findings

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suggest that Ganoderic acid B and Ganoderma polysaccharides suppress A431 cell viability and modulate TGF-β/Smad-related transcripts, which is biologically plausible given the established role of aberrant TGF-β signaling in cSCC progression and therapy resistance. Radiation therapy is known to activate the TGF-β pathway, which can promote DNA repair, EMT, and survival signals in cancer cells, contributing to radioresistance. Our findings suggest that Ganoderma-derived compounds may modulate TGF-β/Smad signaling, a pathway known to mediate cellular responses to radiation.

The pathogenesis of SCC is complex, with driving genes still unclear. Current research has identified related gene pathways, including the Notch, Ras, Epidermal Growth Factor Receptor, Cyclin-dependent kinase inhibitor, and Transforming Growth Factor-β pathways, among others. Among these, TGF-β regulates cell proliferation and differentiation, and the TGF-β/Smad signaling pathway is among the most commonly altered pathways in tumor development [7-9]. Studies have shown that TGF-β1 is an important regulator in the development of squamous epithelial cells and in maintaining tissue homeostasis. Overexpression of TGF-β1 inhibits the formation of papillomas in early stages and promotes the progression, metastasis and epithelial-mesenchymal transition of skin cancer in later stages. Some researchers have found widespread aberrant expression of TGF-β1, TGF-βRI, and Smad7, proteins related to the TGF-β/Smad signaling pathway, in skin squamous cell carcinoma, suggesting that these proteins and their signaling pathway play roles in the pathological process of squamous cell carcinoma [10-12]. Other studies have shown that skin SCC A431 cells may induce downregulation of TGF-β receptor expression, obstructing TGF-β signal transduction and invalidating its inhibitory effect, disrupting the homeostasis of the epidermal environment, leading to the loss of tumor suppressor gene function in skin tumor cells, activation of oncogenes, and ultimately leading to tumor formation [13, 14].

Our findings are also consistent with the broader literature on Ganoderma lucidum. Previous studies and reviews have shown that Ganoderma polysaccharides and triterpenoids exert anticancer effects through antiproliferative, pro-apoptotic, anti-metastatic, and immunomodulatory mechanisms, and may synergize with radiotherapy or chemotherapy in other tumor models. For example, Ganoderma lucidum polysaccharide has been reported to enhance radiosensitivity in hepatocellular carcinoma cells through Akt-related signaling, and recent reviews indicate that Ganoderma-derived compounds may influence oxidative stress, apoptosis, and DNA damage repair pathways [15-18]. In line with our findings, a recent study in an electromagnetic field-exposed rat model demonstrated that oral administration of Ganoderma lucidum extract significantly ameliorated radiation-induced damage to sperm quality and morphology, further supporting the radioprotective and restorative potential of this medicinal mushroom [19, 20].

GDF11, a member of the TGF-β superfamily, is a secretory protein transported and stored in vesicles such as lipid droplets, inclusions, lysosomes, and peroxisomes [21, 22]. GDF11 plays an important role in the development and repair after injury of organisms and various organs by regulating cell proliferation, differentiation, adhesion, migration and apoptosis, and is also involved in various pathological processes [22-24]. Like other TGF-β members, the Smad signaling pathway is the classical signaling pathway for GDF11. Although its role in cancer remains controversial and context-dependent, emerging evidence suggests that GDF11 may participate in tumor progression in several malignancies. However, its role in cSCC is still poorly understood. Therefore, the observed modulation of GDF11 expression in this study may provide preliminary insight into its potential involvement in cSCC biology. However, current domestic and international research on the correlation between the expression level of GDF11 and skin SCC is still in its infancy, and there is a lack of research on the effects of Ganoderma extracts on skin SCC [25].

Importantly, the present study extends these observations to cutaneous squamous cell carcinoma cells and focuses specifically on the TGF-β/Smad axis, which has been strongly linked to cSCC aggressiveness and therapy resistance. In this context, our data provide preliminary evidence that Ganoderma-derived compounds may modulate pathways associated with radiation response [26, 27].

Although the potential interaction between Ganoderma-derived compounds and radiation remains of interest, further studies are required to clarify whether these compounds can enhance radiation response in cSCC. This study has several limitations. First, the experiments were conducted in a single cell line, which may limit generalizability. Second, the interaction between Ganoderma-derived compounds and radiation was not fully characterized. Third, these findings are based on in vitro experiments and require further validation in in vivo models.

CONCLUSION

In summary, Ganoderic acid B and Ganoderma polysaccharides inhibited A431 cell proliferation and were associated with downregulation of TGF-β/Smad -related genes. These findings highlight the potential relevance of Ganoderma-derived compounds in cSCC and warrant further investigation to clarify their role

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in radiation response and therapeutic applications.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical consideration: This study was performed using the A431 cell line and did not involve human participants or animal subjects. Therefore, formal ethical approval was not required.

Author contribution: Conceptualization: [Y.Z., H.T.]. Methodology: [W.X., P.H.]. Investigation: [Y.Z., G.Z.]. Data analysis: [Y.W.]. Writing-original draft, draft review and editing [R.G., H.T]. All authors approved the final manuscript.

Use of AI: The authors declare that AI tools were not used for the generation of scientific content, data interpretation, or final manuscript preparation. AI only used for language editing under human supervision.

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