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# Tumour cell adhesion and integrin expression affected by *Ganoderma lucidum*

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## Abstract

*Ganoderma lucidum* has been widely used as a miraculous herb for health promotion, especially by cancer patients. It has been known that *G. lucidum* affects cancer cell activities. We examined the effect of different preparations of *G. lucidum* spores on malignant human breast carcinoma cell adhesion by treating monolayers of cells with *G. lucidum*. Our experiments indicated that *G. lucidum* inhibited cancer cell adhesion to different degrees, which were: sporoderm-broken spores (broken by an enzymatic method) > sporoderm-broken spores (broken by a physical method) > intact spores > buffer control. Similarly, polysaccharides from different *G. lucidum* sources were used to treat breast carcinoma cells, and its effect on cell adhesion was determined. Polysaccharides isolated from *G. lucidum* fruiting bodies grown on logs of wood exhibited the greatest inhibitory activity on cell adhesion, an effect that was concentration-dependent. Purified polysaccharides also inhibited cell adhesion to various matrix molecules. Experiments were also done where cells were inoculated on polysaccharide-coated Petri plates. This increased cell adhesion, as compared with BSA and PBS controls, suggests that the polysaccharide interacted with cell surface proteins. Western blot analysis indicated that β1-integrin expression was greatly reduced, while β-actin expression was not affected, suggesting a specific activity of *G. lucidum* products on β1-integrin.

## 1. Introduction

Natural products have attracted extensive attention in drug discovery and development in recent years. One of the natural products that has been widely used is *Ganoderma lucidum*. For hundreds of years, this medicinal mushroom has been used in traditional Chinese or folk medicine for the prevention and treatment of various human diseases. More recently, *G. lucidum* has been widely used by patients with different types of cancers in late stages, when it becomes too advanced for surgery, chemotherapy, or radiotherapy, especially in Oriental countries.

For those who have gone through one of these therapeutic treatments, *G. lucidum* is used as a supplement or for an alternative therapy. Although different individuals respond to *G. lucidum* differently, the outcome is generally praise-worthy. Due to its promising effects, studies of *G. lucidum* in tumour biology have been extensive.

It has been reported that the polysaccharides of *G. lucidum* significantly improve the immune parameters of patients with advanced cancers [1]. Researchers have also demonstrated that *G. lucidum* strongly inhibits the migration of breast cancer cells and prostate cancer cells, suggesting its potential to reduce tumour invasiveness [2,3] The polysaccharides of *G. lucidum* can suppress the activity of colon cancer cells and seems to act as a potent chemopreventive agent for colon carcinogenesis [4,5]. The anti-tumour activities of the polysaccharides appear to be due to promotion of the expression of TNFα and IFNγ [6]. It can also suppress tumour-induced angiogenesis [7]. *G. lucidum*'s

anti-cancer effects appear to occur through multiple mechanisms [8]. A recent study has indicated that the sporoderm-broken spores of Ganoderma have much higher bioactivities than the whole spore, and that tumour inhibition reaches 80–90% [9]. This may be due to the presence of the glucan complex [10,11], as it has been shown that the expended chains of sulfated glucan possess higher anti-tumour activity [12].

Since cell adhesion is of primary importance in tumour formation, growth, invasion, and metastasis, we designed experiments to examine whether *G. lucidum* affects tumour cell adhesion in a human breast cancer cell line. We demonstrated that *G. lucidum* inhibited cancer cell adhesion. The polysaccharides isolated from *G. lucidum* cultivated on logs of wood exhibited the greatest activity, an effect that was concentration-dependent. Western blot analysis indicated that expression of β1-integrin was greatly reduced.

## 2. Materials and methods

### 2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Hank's balanced salt solution (HBSS), and trypsin/EDTA were purchased from Invitrogen (Burlington, Ont., Canada). The ECL Western blot detection kit was from Amersham Life Science. Antibodies against β1-integrin and β-actin were from Santa Cruz. Horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody was from Sigma (St. Louis, MO). Tissue culture plates were from Nunc Inc. All chemicals were from Sigma. Jurkat cells were obtained from the American Type Culture Collection (Rockville, MD).

High-quality *G. lucidum* spores and the fruiting body (the mushroom) of *G. lucidum* were identified and selected for use by experts from the Center for Research and Development of Edible Fungi, Guangdong Institute of Microbiology, Guangdong Academy of Sciences (Guangzhou, China). The sporoderm of *G. lucidum* spores were broken using both physical methods and enzymatic methods. The enzymatic method was conducted by inoculating the heat-treated *G. lucidum* spores with *G. lucidum* mycelia, which were obtained from germinating spores. The mycelia released different types of enzymes which digested the sporoderm of the spores. In this way, the bioactive components were slowly released.

To extract polysaccharides, *G. lucidum* fruiting bodies were smashed to small pieces, and were then incubated in hot water for 2 h. The solution was subjected to centrifugation to remove particles, concentrated to a small volume, and dried to powder. This is the total polysaccharide. The polysaccharides were also subjected to further purification by ethanol (75%) precipitation. The concentrated *G. lucidum* polysaccharide solution was precipitated by addition of ethanol to a final concentration of 75% (v/v) ethanol. The precipitated pellet was resuspended in water followed by ethanol precipitation. This procedure was repeated six times to remove the small molecules from the preparation. This polysaccharide was called purified polysaccharide. These procedures were performed at the Center for Research and Development of Edible Fungi, Guangdong Institute of Microbiology. The commercial products of *G. lucidum* were also purchased from the Greater Toronto Area.

### 2.2. Cell adhesion assay

Human breast malignant carcinoma cells (MT-1), maintained in a monolayer culture on tissue culture plates in 1 ml Dulbecco's modified Eagle's medium (DMEM) containing penicillin, streptomycin, and 10% fetal bovine serum (FBS), were used in our studies. The cells were seeded on 12-well tissue culture plates at a density of $1 \times 10^5$ cells per well. The isolated components, the total extract of sporoderm-broken spores, and the intact spores of *G. lucidum* were added to the cultures. The buffer used to dissolve the *G. lucidum* products served as a control. The cultures were incubated at 37 °C for 12 h, after which unattached cells were removed and the plates were washed. The attached cells were then fixed and stained with Coomassie blue for easier examination of cell attachment. The stained cells were examined under a light microscope and photographed. The number of cells per microscopic field was counted for a minimum of 10 fields. These techniques are routinely used in our laboratory [13–15].

### 2.3. Polysaccharide-coating experiment

The polysaccharides of *G. lucidum* dissolved in PBS (20 mg/ml) were used to coat Petri dishes at 37 °C for 2 h. Controls were bovine serum albumin (BSA) dissolved in PBS (20 mg/ml) or PBS alone. After removing the coating agents, human breast carcinoma cells were inoculated onto the plates, followed by incubation at 37 °C for 30 min. Unattached cells were removed and the plates were washed. The attached cells were harvested and the number of cells was counted.

### 2.4. Protein extraction and Western blot analysis

The Western blot procedure was performed as previously described [16–18]. Briefly, after treatment with polysaccharides, cells were washed twice with ice-cold PBS and lysed with RIPA buffer (50 mM Tris–HCl, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate and 0.1% SDS) containing 1 mM DTT, 1 mM $Na_3VO_4$, 5 mM NaF, 100 mM EDTA, 10 mg/ml aprotinin, and 100 mM PMSF. Protein samples were subjected to SDS-PAGE on separating gel containing 10% polyacrylamide in reducing loading dye (1×) containing 50 mM Tris–Cl, pH 7.2, 2% SDS, 10% glycerol, and 0.02% bromophenol blue. The buffer system was 1×-Tris/glycine buffer (Amresco) containing 1% SDS. Separated proteins were transblotted onto a nitrocellulose membrane (Bio-Rad) in 1× TG buffer (Amresco) containing 20% methanol. The membrane was blocked in TBST (10 mM Tris–Cl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing 10% non-fat dry milk powder (TBSTM) for 1 h at room temperature, and then incubated at 4 °C overnight with primary antibody prepared in TBSTM. The membranes were washed with TBST (3 × 30 min) and then incubated for 1 h in TBSTM with goat anti-mouse secondary antibody conjugated to horseradish peroxidase. After being washed as described above, the bound antibody was visualized with an ECL kit according to the manufacturer's instructions.

### 2.5. Statistical analysis

Differences among treatment means were determined by Student's *t*-test. $p < 0.05$ was considered significant.

## 3. Results and discussion

### 3.1. Ganoderma lucidum products inhibit cancer cell adhesion

*Ganoderma lucidum*, a popular medicinal mushroom, has been widely used as a miraculous herb for health promotion in Oriental countries. The polysaccharides and ganoderic acid are the major components of biological activity, which possess anti-tumour and anti-HIV-1 activities, and are of therapeutic use. Researchers have also demonstrated that *Ganoderma lucidum* strongly inhibits properties in the migration of breast cancer cells and prostate cancer cells, suggesting its potency to reduce tumour invasiveness [3]. The polysaccharides of *G. lucidum* improve the immune parameters of patients with advanced cancers and promotes expression of TNFα and IFNγ. *G. lucidum* appears to work through multiple mechanisms, which underlie its anti-cancer effects [8]. We hypothesize that *G. lucidum* also affects cancer cell adhesion.

The interaction of cells with their surrounding matrix and neighbouring cells governs many aspects of cell behaviour.

Cell–cell adhesion determines the polarity of cells and participates in the maintenance of the cell societies called tissues. Adhesion is generally reduced in human cancer cells [19,20]. Reduced intercellular adhesion allows cancer cells to disobey the social order, resulting in the destruction of histological structure, which is the morphological hallmark of malignant tumours [20]. Reduced intercellular adhesiveness is also indispensable for cancer invasion and metastasis. However, it is also well known that cell adhesion is essential for anchorage-dependent cell growth [21,22]. Adhesion receptors such as integrins provide mechanical support and survival signals [22].

We first obtained a large number of *G. lucidum* products from the Greater Toronto Area and examined the products under a light microscope. We also compared the *G. lucidum* products to be used in our studies (provided by the Center for Research and Development of Edible Fungi, Guangdong Institute of Microbiology) with those purchased from the market in Toronto. These products were imported from China (Mainland and Hong Kong), Japan, the United States, and New Zealand, or produced in Canada. The products from Guangdong Institute of Microbiology were mainly sporoderm-broken spores and intact spores of *G. lucidum*, while those purchased from Toronto were the powdered fruiting body of *G. lucidum*, mycelia (sprouting spores), and some intact spores of *G. lucidum* (Fig. 1).

We developed a cancer cell culture model to study the effect of *G. lucidum* on cell activities. Using the malignant human breast carcinoma cell line MT-1 [23], maintained in a monolayer culture on tissue culture plates, we examined the effect of the *G. lucidum* products described above on cancer cell adhesion. The *G. lucidum* products were suspended in PBS (20 mg/ml) and incubated at $100\,^{\circ}$C for 60 min. The preparations were then added to the cell cultures (50 μl/well, 1 mg/ml), followed by incubation at $37\,^{\circ}$C for 12 h. Addition of PBS alone served as a control. The effect of different *G. lucidum* products on tumour
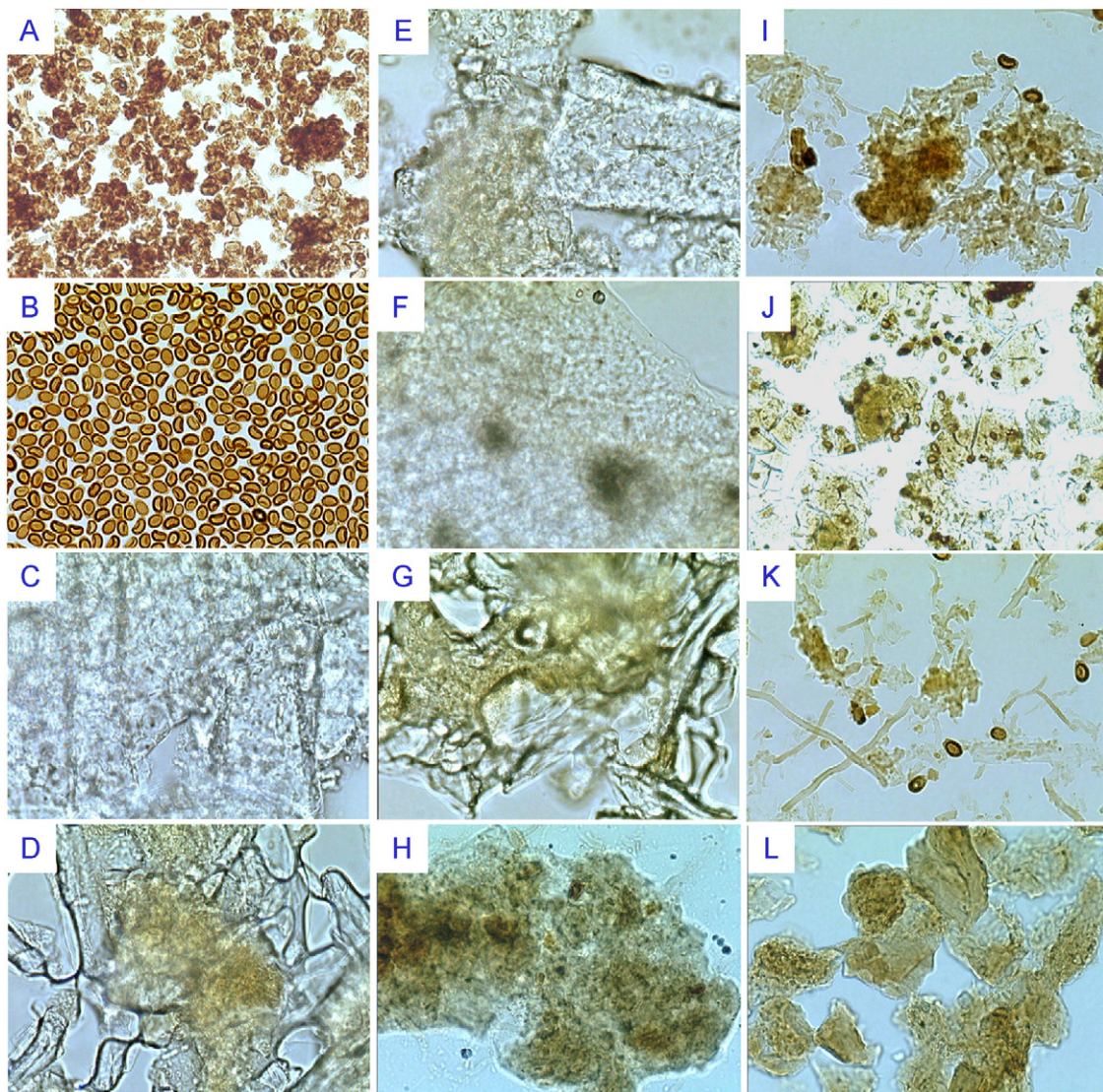


Fig. 1. Comparison of *G. lucidum* products. (A) Sporoderm-broken spores of *G. lucidum*; (B) intact spores of *G. lucidum*; (C–H) powdered fruiting body of *G. lucidum*; (I–K) mycelia, fruiting body and some intact spores of *G. lucidum*; (L) precipitate of extract from the fruiting body of *G. lucidum*. (A and B) Obtained from the Center for Research and Development of Edible Fungi, Guangdong Institute of Microbiology. (C–L) Purchased from stores in the Greater Toronto Area.
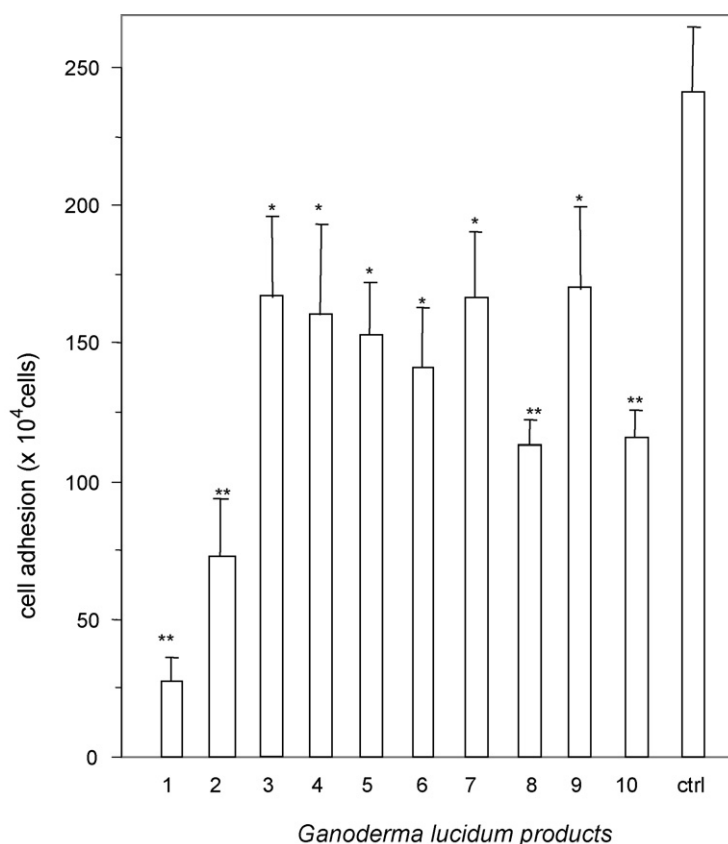
Fig. 2. The effect of different *Ganoderma lucidum* products on cell adhesion. Malignant human breast carcinoma cells (MT-1), maintained in a monolayer culture on tissue culture plates in 1 ml medium containing 10% FBS, were used in our studies. A large number of *G. lucidum* products was obtained from the market in Toronto. These products were imported from China (Mainland and Hong Kong), Japan, the United States, Canada, and New Zealand. The *G. lucidum* products were suspended in PBS (20 mg/ml), followed by incubation at 100 °C for 60 min. *G. lucidum* products of equal concentration were added to carcinoma cell cultures (0.5 mg/ml), followed by incubation at 37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed and stained with Coomassie blue for microscopic examination and cell counting. Each bar represents one commercial product. Buffer vehicle alone served as a control (ctrl). (1) Polysaccharides isolated from fruiting body cultivated on logs of wood; (2) Lucid Ganoderma from M&A Pharmaceutical Factory Co. Ltd., Japan; (3) Wild-Lingzhi from Well Herbs Nutrition Ltd., Fendalton Christchurch, New Zealand; (4) Arashi Kuni Dual-Breakage Ganoderma Spores from Natural Corporation Ltd., Hong Kong; (5) The Original Premium Lingzhi from PuraPharm International Ltd., Hong Kong; (6) Vita Green Lingzhi from Vita Green Pharmaceutical Ltd., Hong Kong; (7) Mikei Reishi from Nikkei Co., Sawa-Gun, Gumma-Ken, Japan; (8) Japan *Ganoderma Lucidum* Powder from Tokyo Herb Co. Ltd., Tokyo, Japan; (9) Lingzhi Master from Care & Health Ltd., Hong Kong; (10) Extra Strength Lingzhi Craked Spores from Eu Yan Sang Ltd., Hong Kong.

cell adhesion was examined. Our experiments indicated that once treated with *G. lucidum* products, the tumour cells exhibited a decrease in cell adhesion (Fig. 2). However, this decrease was variable, and the best results were obtained by using the sporoderm-broken spores of *G. lucidum*.

It should be noted that since the *G. lucidum* products inhibit tumour cell proliferation and induce tumour cell death, these effects were also observed in our results. However, we also observed that the decrease in cell proliferation and increase in cell death were later events, and that the effect of the *G. lucidum* products on cell adhesion was an early event. As such, we were able to obtain good results of reduced cell adhesion within 12 h, but it was appropriate to examine cell growth and cell death after 24 h of treatment.

### 3.2. Effect of G. lucidum spores on cancer cell adhesion

The results we obtained using sporoderm-broken spores and intact spores of *G. lucidum* were consistent with published

papers. It has been known that the spores of *G. lucidum* work better in disease treatments than the fruiting body does [24], while the sporoderm-broken spores have higher bioactivities than the whole spores [9]. The bioactive substances in the spores are not well utilized in vivo when the sporoderm is not broken. The whole spores have been found in the dejecta of animals and humans who took the whole spores during treatments [9].

We therefore examined the effect of the sporoderm-broken spores of *G. lucidum* on cancer cell adhesion. The sporoderm-broken spores were generated using either a physical method or by enzymatic digestion. The latter allows slow digestion of the spores, resulting in the release of bioactive components. Malignant human breast carcinoma cells were maintained as monolayer cultures on tissue culture plates as above. Intact *G. lucidum* spores and sporoderm-broken spores were suspended in PBS (20 mg/ml), then incubated at 100 °C for 60 min. The samples were added to the cell cultures (0.5 mg/ml), which had been seeded on plates for 30 min, followed by incubation at
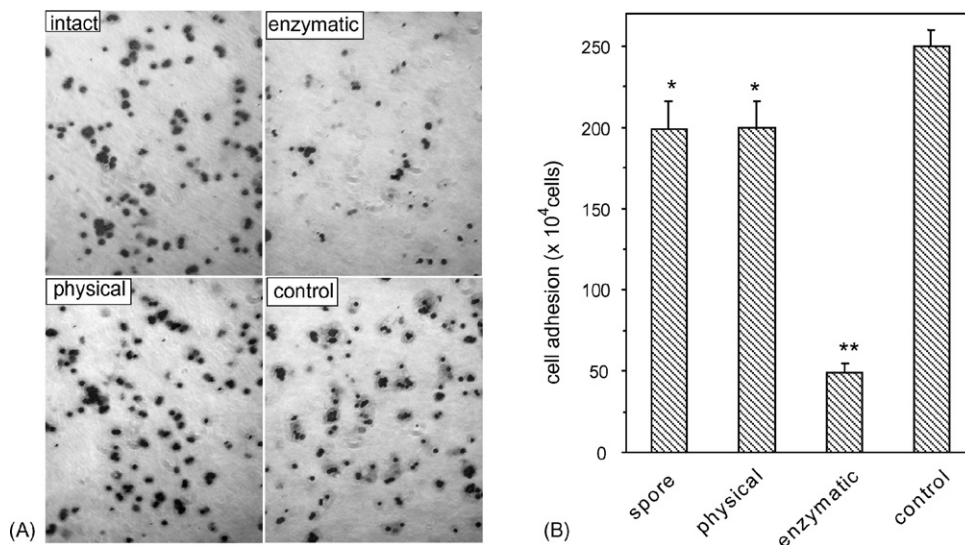
Fig. 3. The effect of sporoderm-broken spores generated by different methods on cell adhesion. Cell adhesion is an important step in the initiation of tumour formation. We tested the effects of different preparations of *G. lucidum* spores on cancer cell adhesion. Malignant human breast carcinoma cells were incubated with *G. lucidum* spores and sporoderm-broken spores suspended in PBS (0.5 mg/ml) at 37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed and stained with Coomassie blue for microscopic examination (A) and cell counting (B). Our experiments indicated that *G. lucidum* inhibited cancer cell adhesion, and the inhibitory effects were: sporoderm-broken spores (enzymatic method) > sporoderm-broken spores (physical method) = intact spores > buffer control.

37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed and stained with Coomassie blue for microscopic examination and cell counting. Our experiments indicated the inhibitory effects on cancer cell adhesion were sporoderm-broken spores (enzymatic method) > sporoderm-broken spores (physical method) = intact spores > buffer control (Fig. 3A), which was statistically significant (Fig. 3B). Our study suggests that breaking the sporoderm of *G. lucidum* spores is essential to improve the bioactivity of the *G. lucidum* products. Furthermore, the method used to break the sporoderm are also important. Enzymatic treatment allows the sporoderm to be digested and the bioactive components to be released. However, it should be pointed out that extensive digestion would cleave the bioactive components, which should be avoided.

### 3.3. Interaction of polysaccharide with tumour cells

The major bioactive components in *G. lucidum* are polysaccharides, ganoderic acid, and adenosine, of which the polysaccharides are the major source of its biological activity and therapeutic use [25–28]. We tested whether the polysaccharides could affect tumour cell adhesion. Polysaccharides were prepared from different sources, including the fruiting body grown in wood logs, mycelia, and wild-type fruiting body obtained from the mountains in South China. Human malignant breast carcinoma cells were grown as monolayer cultures, to which polysaccharides of *G. lucidum* were added at a final concentration of 0.5 mg/ml, followed by incubation at 37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed and stained with Coomassie blue for microscopic examination and cell counting. The experiments indicated that the polysaccharides inhibited cancer cell adhesion, and the

inhibitory effects were wood log(1) > mycelium (2) > wild type (3) > buffer control (Fig. 4A), all of which were of statistical significance (Fig. 4B). The inhibitory effect of polysaccharides (from fruiting bodies grown in logs of wood) on cancer cell adhesion was concentration-dependent (Fig. 4C). Although the polysaccharides were analyzed by gel electrophoresis and Coomassie blue staining, and no protein contamination was detected, we could not exclude the possibility of contamination by small molecules. To avoid this, we have purified the polysaccharides by repeating ethanol purification six times and tested the activity of the purified polysaccharides in the reduction of cell adhesion.

Human leukemia cells (Jurkat) were inoculated on tissue culture plates, which had been coated with different extracellular matrix molecules including type I collagen, hyaluronan, fibronectin, and laminin. Coating with bovine serum albumin served as a control. *G. lucidum* has been reported to inhibit cancer cell adhesion to some of these matrix molecules [2]. The purified polysaccharides were dissolved in PBS and added to the cultures at a final concentration of 0.4 mg/ml, followed by incubation at 37 °C for 12 h. The unattached cells were removed the following day, and the plates were washed. The attached cells were fixed for cell counting (Fig. 5A). Typical pictures of cell attachment are shown (Fig. 5B). The experiments indicated that the polysaccharides inhibited adhesion of Jurkat cells to these matrix molecules. Our results are in agreement with previous reports that *G. lucidum* reduces cancer cell adhesion to the extracellular matrix [2]. Previous work has also indicated that *G. lucidum* also suppresses tumour cell motility [3], which is an important characteristic of tumour invasion. Since cell adhesion is essential for a migrating cell to survive in a new environment, the role of *G. lucidum* in reduceing cell adhesion and motility imply that *G. lucidum* can inhibit tumour invasion.

To investigate how the polysaccharides of *G. lucidum* affected tumour cell adhesion, we tested whether the polysaccharides could bind to the cell surface. The polysaccharides of *G. lucidum* were dissolved in PBS at a concentration of 20 mg/ml. The solution was then used to coat Petri dishes at 37 °C for 2 h. Controls were bovine serum albumin (BSA) dissolved in PBS (20 mg/ml), or PBS alone. The rationale for using Petri dishes was that the cancer cells used do not attach very well to the plates. If the cells bound to the polysaccharides, we would be able to detect cell attachment after incubation. After removing the coating solution, human breast carcinoma cells were inoculated onto the plates, then incubated at 37 °C for 30 min. Unattached cells were removed and the plates were washed with PBS. Cell attachment was examined under a light microscope and photographed. The attached cells were also harvested and the cell number was counted. A large number of cells attached to the polysaccharide-coated plates (Fig. 6A), which was statistically significant as compared to plates coated with BSA and PBS alone (Fig. 6B). Our experiments indicated that that the breast cancer cells interacted with the polysaccharides of *G. lucidum*. Isolation of the cell surface binding proteins is underway.

### 3.4. Effect of polysaccharide of G. lucidum on β1-integrin expression

Cell adhesion is an important step in the initiation of tumour formation. It is associated with tumour cell invasion and metastasis. Researchers have also demonstrated that *G. lucidum* strongly inhibits the migration of breast cancer cells and prostate cancer cells, suggesting its potency to reduce tumour invasiveness
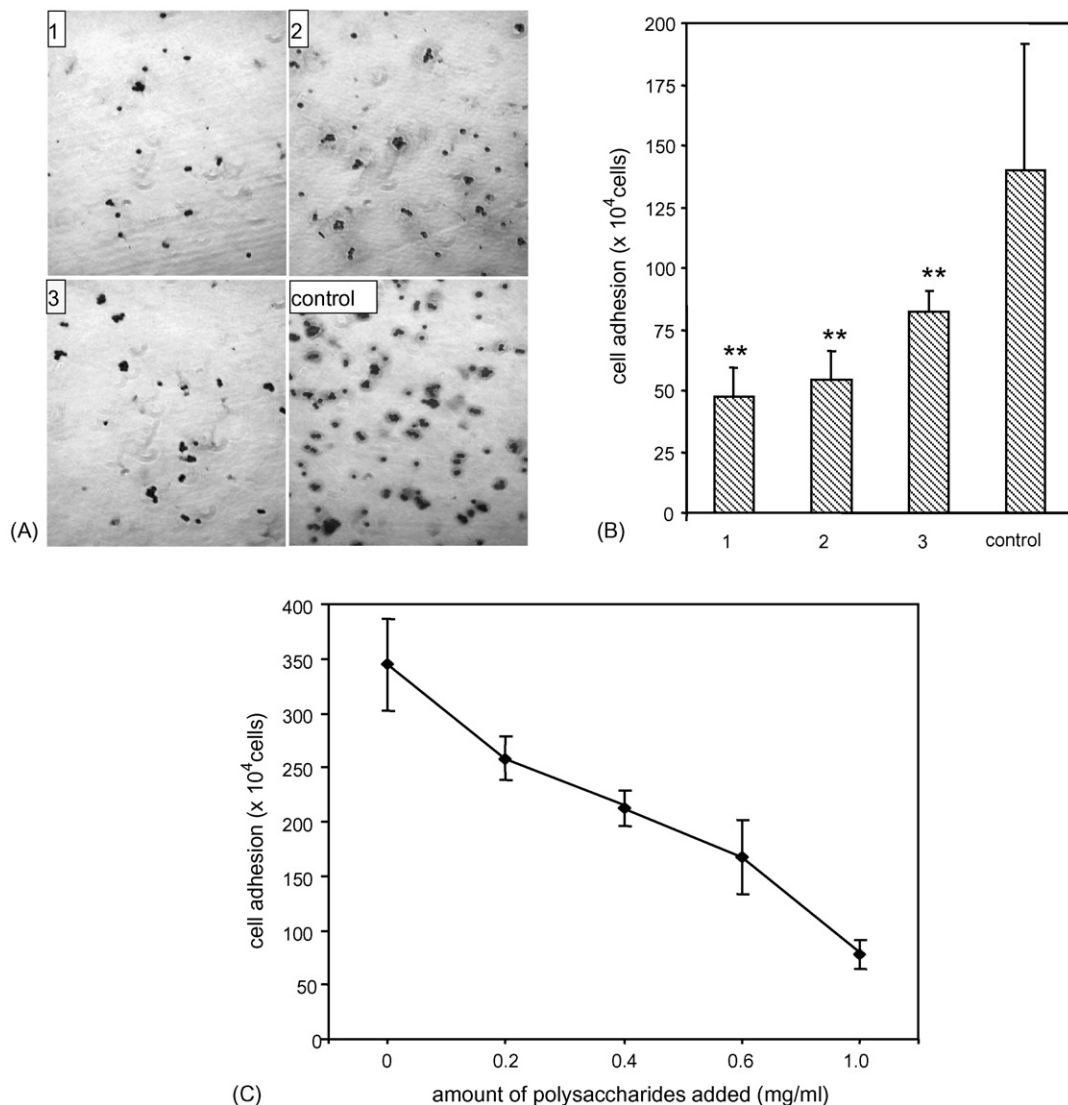


Fig. 4. The effect of polysaccharides from different sources on tumour cell adhesion. Malignant human breast carcinoma cells were maintained in a monolayer culture, to which polysaccharides of *G. lucidum* obtained from different sources were added at a final concentration of 0.5 mg/ml, followed by incubation at 37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed and stained with Coomassie blue for microscopic examination (A) and cell counting (B). The experiments indicated that the polysaccharides inhibited cancer cell adhesion, and the inhibitory effects were as follows: wood log (1) > mycelium (2) > wild type (3) > buffer control ($n = 3$, $^{**}p < 0.01$). The inhibitory effect of polysaccharides (from wood log) was concentration-dependent (C).

[2,24]. Our results showing that *G. lucidum* affect cancer cell adhesion therefore appear to be of physiological relevance. As such, we further investigated how *G. lucidum* polysaccharides might affect cancer cell adhesion. Since integrins are the major cell surface adhesion molecules expressed by all cell types, we examined whether incubation with *G. lucidum* polysaccharides reduced integrin expression. Integrins are composed of α and β transmembrane subunits [29]. Each αβ combination has its own binding specificity and signaling properties. Incorporated with various α subunits, β1-integrin binds to diverse extracellular molecules [30]. The fundamental cellular function of integrins is adhesion, and they mediate extensive and important cellular functions by interacting with the extracellular matrix [31], a process which activates signal transduction. It is well known that

β1-integrin is expressed by all types of cells and associated with cell adhesion [22,32–34]; we therefore analyzed β1-integrin expression after the cells were treated with *G. lucidum* polysaccharides.

Malignant human breast carcinoma cells were incubated with the polysaccharides of *G. lucidum* suspended in PBS (0.5 mg/ml) at 37 °C for 24 h. The cells were lysed and equal amounts of cell lysate were separated on SDS-PAGE, followed by Western blotting probed with anti-β1-integrin monoclonal antibody. The same samples were also analyzed on Western blot and probed with anti-actin antibody to assess equal loading. Cells treated with the polysaccharide expressed a lower level of β1-integrin compared to the untreated cells (Fig. 7A). Little difference was detected in actin expression between the cells treated with the
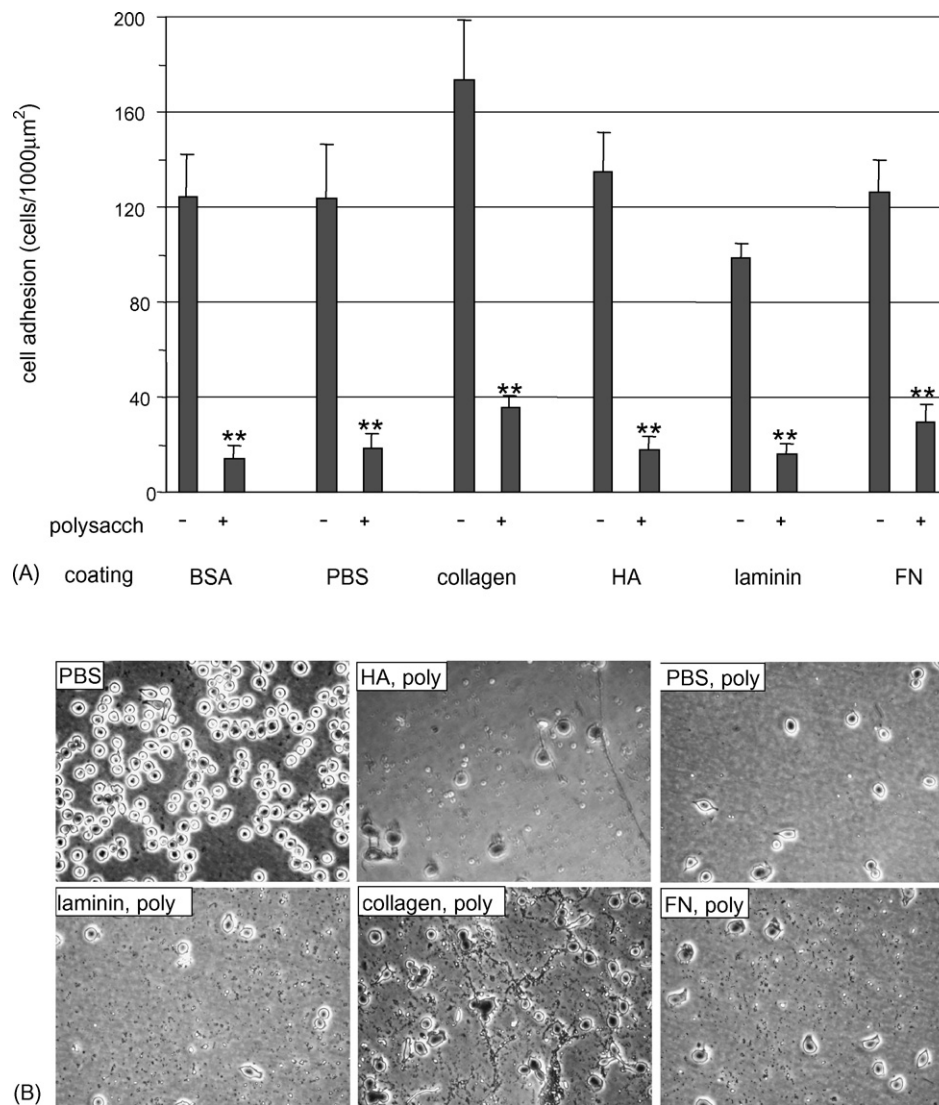


Fig. 5. The effect of polysaccharides on tumour cell adhesion to other matrix molecules. Tissue culture plates (24-well) were coated with 80 µl of type I collagen (0.2 mg/ml), hyaluronan (HA, 0.5 mg/ml), fibronectin (FN, 20 ng/ml), laminin (20 µg/ml), bovine serum albumin (BSA, 1% in PBS), and PBS at 4 °C overnight. The coating solution was removed, followed by inoculation of 0.5 ml human leukemia cells (Jurkat) at a cell density of $5 \times 10^5$ cells/ml. The cultures were maintained at 37 °C for 30 min. Polysaccharides, which were purified by repeatedly ethanol precipitation for six times, were dissolved in PBS (20 mg/ml) and added to the cultures at a final concentration of 0.4 mg/ml, followed by incubation at 37 °C for 12 h. Unattached cells were removed and the plates were washed. The attached cells were fixed. Cell numbers were counted (A). Typical pictures for cell attachment are shown (B). The experiments indicated that the polysaccharides inhibited adhesion of Jurkat cells.
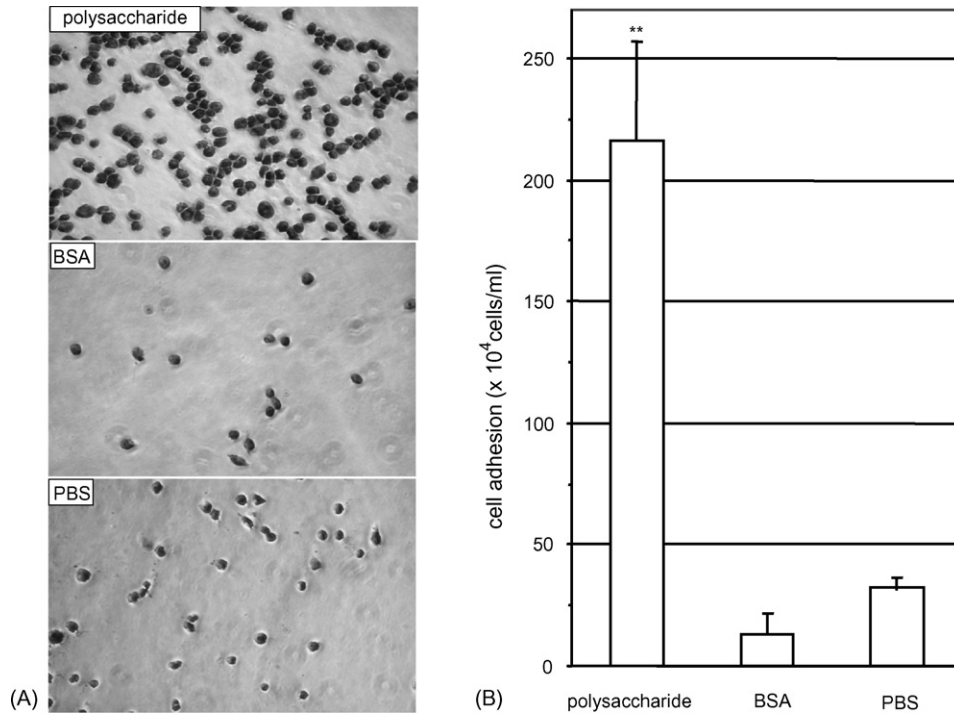
Fig. 6. The interaction of polysaccharides with tumour cells. The polysaccharides of *G. lucidum* dissolved in PBS (20 mg/ml) was used to coat Petri dishes at 37 °C for 2 h. Controls were bovine serum albumin (BSA) dissolved in PBS (20 mg/ml) or PBS alone. After removing the coating agent, human breast carcinoma cells were inoculated onto the plates, followed by incubation at 37 °C for 30 min. Unattached cells were removed and the plates were washed (A). The attached cells were harvested, and the cell number was counted (B). A large number of cells attached to the polysaccharide-coated plates, suggesting that the cells interact with the polysaccharides.
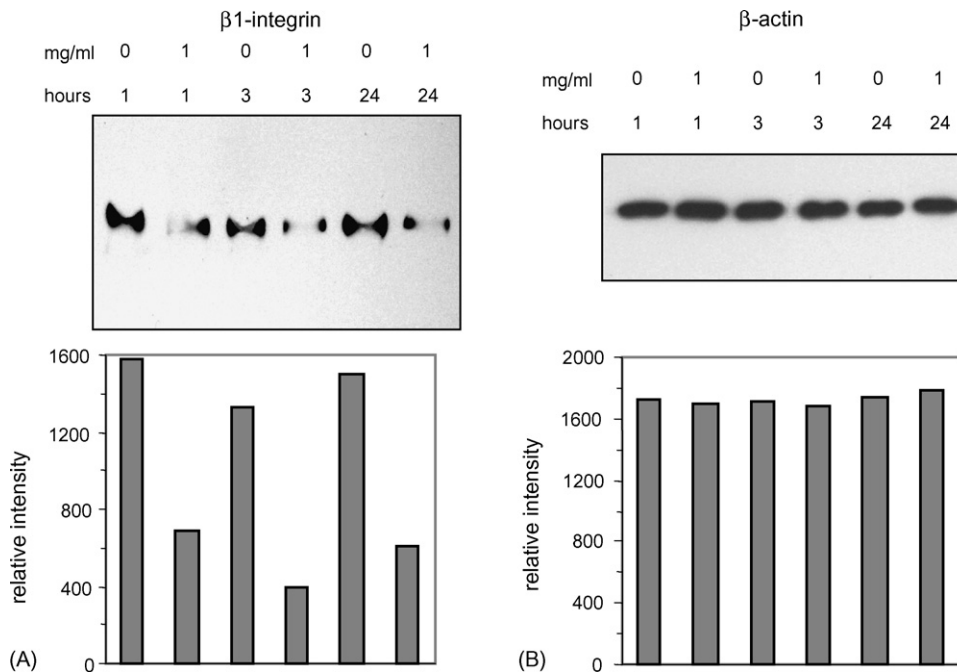


Fig. 7. The effect of the polysaccharides of *G. lucidum* on β1-integrin expression. Malignant human breast carcinoma cells were incubated with the polysaccharides of *G. lucidum* suspended in PBS (0.5 mg/ml) at 37 °C for 2 days. The cells were lysed and equal amounts of cell lysate were separated through SDS-PAGE using a 7% SDS gel, followed by Western blot analysis probed with anti-β1-integrin monoclonal antibody (A). The same samples were also analyzed on Western blot and probed with anti-actin antibody as a loading control (B). Cells treated with the polysaccharides expressed a lower level of β1-integrin compared to the untreated cells. Little difference was detected in actin expression between the cells treated with the polysaccharides and those that were untreated.

polysaccharides and the untreated cells (Fig. 7B). Thus, we have demonstrated that a cell adhesion molecule is found to be involved in *G. lucidum* inhibition of cell adhesion. However, the molecular mechanism is not clear. We do not know whether *G. lucidum* directly down regulated β1-integrin expression, or whether *G. lucidum* reduced cell adhesion resulting in the reduction in β1-integrin expression. This awaits further investigation.

In summary, we have for the first time established a cell model to test the effect of *G. lucidum* on cell adhesion, and have observed that *G. lucidum* products reduced cancer cell adhesion. Furthermore, we have shown that the polysaccharides of *G. lucidum* directly interacted with cells, possibly by binding to cell surface proteins. Finally, we have demonstrated that cancer cells incubated with the polysaccharides of *G. lucidum* exhibited a reduced level of β1-integrin expression. To determine whether there is a link between polysaccharide binding to the cell surface and a decrease in β1-integrin expression requires further investigation.

## 4. Conclusion

We examined the role of *G. lucidum* in cancer cell adhesion and demonstrated that *G. lucidum* products reduced tumour cell adhesion. The sporoderm-broken spores of *G. lucidum* produced a greater effect on cancer cell adhesion than intact *G. lucidum* spores, and those generated by enzymatic method produced better results than the ones generated by enzymatic digestion. The polysaccharides seemed to be the major component in reducing tumour cell adhesion, and those obtained from fruiting bodies grown in wood logs produced the best result. The inhibitory effect of *G. lucidum* on cell adhesion is mediated through the integrin pathway.

## Acknowledgements

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