Ganoderma lucidum polysaccharide inhibits the proliferation of leukemic cells through apoptosis

Mingxing Zhong1, Jiwei Huang1, Pingxiang Mao2, Chengming He1, Dongmei Yuan1, Chang-kun Chen1, Haiying Zhang1, Jing Hu1 and Jingdong Zhang1

Objective: To investigate the cytotoxic effect of polysaccharides derived from Ganoderma lucidum on T lymphocyte leukemia cells. Methods: Water-soluble polysaccharides were extracted from the fruit bodies of G. lucidum, purified, and characterized using HPGPC-MALLS and NMR. The cytotoxicity of G. lucidum polysaccharide fraction 5 (GLPS5) to T lymphocyte leukemia cell line Jurkat and human immortalized epidermal cell line HaCaT was assessed using MTT assay. Apoptosis was assessed using flow cytometry. Expressions of apoptosis-related genes in the cells after being exposed to GLPS5 were detected using Western blot assay. Results: GLPS5 was a β-(1→3) and β-(1→6) linked glucan. It inhibited the proliferation of Jurkat cells in a concentration-dependent manner and the half-maximal inhibitory concentration (IC50) was 34.5 mg/L but did not suppress the growth of HaCaT cells. Apoptotic cells in Jurkat cells were detected to increase with increasing GLPS5 concentrations. The expression levels of cleaved caspase-3 were significantly higher after the cells were exposed to 25 and 50 mg/L GLPS5 when compared to non-exposed cells (Control). In addition, the expression levels of BAX and Bcl2 were significantly up- and down-regulated after treatment with GLPS5 at 25 and 50 mg/L when compared with control (P<0.05, respectively). Conclusions: GLPS5 has anti-proliferative activity against Jurkat cells and the activity is likely mediated through the activation of apoptosis pathways.

Keywords: Ganoderma lucidum, polysaccharide, leukemia, Jurkat, cytotoxicity, apoptosis

Received: 07 December, 2021; revised: 28 April, 2022; accepted: 06 May, 2022; available on-line: 28 June, 2022

INTRODUCTION

Leukemia is a group of malignant hematological diseases. Among them, acute myeloid leukemia (AML) is the most common leukemia in adult population with a prevalence rate between 3 and 5 in 100,000 people (Bray et al., 2018; Deak et al., 2021; Ranta et al., 2017). Currently, AML is mainly treated using stem cell transplantation and chemotherapy (Murphy & Yee, 2017; Takami, 2018), which are very expensive and have serious adverse reactions (Bewersdorf et al., 2019). Despite substantial advances in our understanding of AML, patient survival remains unsatisfactory, especially within the older age group. The 5-year survival rate after treatments is less than 50% (Hoseini & Cheung, 2017a; Hoseini & Cheung, 2017b; Maia Rda & Wunsch Filho, 2013). Therefore, there is an urgent need to develop efficient, low in toxicity, economic, and targeted drugs for treatment of leukemias (Boudny & Trbusek, 2020).

The mushroom Ganoderma lucidum is a basidiomycete rot macrofungus with a potent pharmacological value (Sanodiya et al., 2009). Polysaccharides are the main components of G. lucidum and have a wide range of pharmacological activities, such as antibacterial, antitumor, and antioxidant activity (Sanodiya et al., 2009; Sohretoglu & Huang, 2018). The bioactivity of polysaccharides is closely related to their molecular structures and chemical properties, such as molecular weight, sulfate content, water solubility, conformation, and sugar chain type (Li et al., 2013). Low molecular weight polysaccharides from G. lucidum (GLP) have been shown to be cytotoxic to various types of tumors, such as colon cancer, ovarian cancer, and prostate cancer (Amini et al., 2019; Kladar et al., 2016). In addition, polysaccharides from Spirulina platensis (PSP) can increase the activity of natural killer (NK) cells in patients with leukemia but did not affect the activity in healthy people. PSP also increases the activity of lymphokine-activated killer (LAK) cells in patients with leukemia, leading to a reduced use of interleukin (IL)-2 (Zeng et al., 2006a; Zeng et al., 2006b). A fraction of GLP was shown to be able to induce macrophage-like differentiation in human leukemia cells via caspase and p53 activation (Hsu et al., 2011), enabling potential leukemia differentiation therapy. In addition, GPL may exert an anti-tumor effect by stimulating the release of cytokines from activated macrophages (Wang et al., 1997). However, whether GPL has cytotoxicity against leukemia cells is largely unclear.

In the present study, we aimed to investigate the cytotoxicity of GPL to human leukemia cell line Jurkat and examine the possible mechanisms underlying the activity.

Acknowledgements of Financial Support: This study was supported by Provincial Natural Science Foundation, Jiangxi, China (2017BAB205044).

Abbreviations: AML, acute myeloid leukemia; BAX, BCL-2-associated X apoptosis regulator; COX-2, cyclooxygenase-2; CR3, complement receptor; GLPS5, G. lucidum polysaccharide; IC50, half-maximal inhibitory concentration; LAK, lymphokine-activated killer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 3-NC, 2-amino-4-(3-nitrophenyl)-3-cyano-7-(dimethylamino)-4H-chromene NK; natural killer; OD, optical density; p-Akt1, phosphoserine/threonine kinase 1; P-ERK, phospho-extracellular regulated protein kinases; PS, phosphatidylinositol-serine; PSP, polysaccharides from Spirulina platensis; PVDF, polyvinylidene fluoride; RPMI, Roswell Park Memorial Institute; S.D., standard deviation; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; VDAC, voltage-dependent anion channel.

Epub: No 6070
Paper in Press
https://doi.org/10.18388/abp.2020_6070

Regular paper
The findings would help develop potential therapeutic agents for leukemia.

MATERIALS AND METHODS

G. lucidum and cell lines
Dry powder of G. lucidum fruiting bodies was purchased from Tuohai Biotech, Hebei, China. Human acute T cell leukemia cell line Jurkat, clone E6-1 (Cat. no. TIB152) purchased from American Type Collection Center (ATCC), and human immortalized epidermal cell line HaCat purchased from Weipin Biotech, Shanghai, were cultured in RPMI-1640 medium (Cat. no. 11875119, Gibco, USA) with 10% fetal bovine serum (FBS) (Cat. no. 14190-149, Gibco), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO2.

Reagents and instruments
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and sodium dodecyl sulfate (SDS) were purchased from Dingguo Changsheng Biotech, Beijing, China; penicillin and streptomycin mixture was purchased from Biyuntian Biotech, Beijing, China; PVDF membrane was purchased from Sigma, St. Luis, USA; antibodies against cleaved caspase-3 (Cat. no. ab32042, 1:1500), Bax (Cat. no. ab32503, 1:1000), Becl2 (Cat. no. ab32124, 1:2500), and GAPDH (Cat. no. ab253778, 1:1000) were purchased from Abcam, USA; and HRP labeled-goat anti mouse IgG secondary antibody (Cat. no. 32430; 1:2000) and HRP labeled-goat, rabbit IgG secondary antibody (Cat. no. 12460; 1:2000) were purchased from Promega, USA; BCA protein assay (Cat. no. 23227), Dead Cell Apoptosis Kit with Annexin V FITC, and PI (Cat. no. V13242) and ultra-sensitive luminescent solution (Cat. no. RF239676) were obtained from Thermo Fisher, USA. Microplate reader (GloMax Discover) was obtained from Promega, USA; vertical laminar flow cabinet (OptiMair) and inverted fluorescence microscope (Revolve FL) were purchased from Esco Lifesciences, Beijing, China; DEAE-52 and Sephadex G-100 were purchased from Whatman (Maidstone, Kent, UK) and Pharmacia (Sweden); 1260 Infinity II LC System was obtained from Agilent, Santa Clara, USA.

Preparation of GLP
GLP was prepared and characterized as previously reported (Zhao et al., 2010). Briefly, 100g powder was degreased by soaking in 500 ml 95% ethanol for 24 h on a rotary shaker operated at 80 rpm at room temperature. The degreased power was dried at room temperature for 20 min to pellet undissolved debris. The supernatant was vacuum-dried at 4°C for 24 h. The aqueous extract was centrifuged at 500×g for 10 min in the dark at 4°C and loaded onto a flow cytometer (Becton Dickinson FACS) for assessment.

Flow cytometry apoptosis assays
Flow cytometry analyses were used to assess apoptotic cells after double staining with FITC-Annexin V and PI included in the Dead Cell Apoptosis Kit according to the supplier’s instructions. HaCat and Jurkat cells were cultured for 48 h with indicated concentrations of GLP. The cells (both treated and untreated) were harvested and rinsed twice with PBS, then resuspended in 100 μl binding buffer included in the kit. 10 μl FITC-Annexin V was added to the cells followed by the addition of 10 μl PI. The samples were then incubated for 10 min in the dark at 4°C and loaded onto a flow cytometer. The data were analyzed for gray value with the “Image Lab” software.

Determination of relative molecular mass and molecular radius
High-performance gel permeation chromatography-multi-angle laser light scattering (HPGPC-MALLS) was used to determine the relative molecular mass and molecular radius. The liquid chromatography contained a SB-806HQ Shodex OH pak column (300 mm×8 mm, 6 μm) and a mobile phase of 0.1mol/l Na2SO4 flowing at 0.6 ml/min. The column temperature was set at 35°C with 100 μl injection volume filtered through a membrane of 0.22 μm.

NMR spectrum analysis
20 mg GLP was dissolved in 500 μl D2O and frozen-dried three times. It was then dissolved in 500 μl D2O and analyzed at Biotech-pack Inc., Beijing on an Agilent DD 500 MHz superconducting NMR spectrometer to obtain NMR spectrums for 1H and 13C, 1H-1H COSY spectrums and 1H-13C HSQC spectrums at 25°C. Deuterated DMSO was used as an internal standard.

In vitro cytotoxicity assay
HaCat and Jurkat cells in the logarithmic growth phase after the third passage in RPMI medium containing 10% FBS were used for the experiments. The cells were inoculated into RPMI medium containing different concentrations of GLP (0 to 1000 mg/l) in the wells of 96 well plates and cultured at 37°C and 5% CO2 for 48 h. 20μl MTT (5 g/l) was added, and the cells were cultured for another 4 h before being used for optical density (OD) assessment at 570 nm according to the manufacturer’s instructions.

Western blot
500 μL Jurkat cells (2×106 cells/mL) in the logarithmic growth phase were cultured in RPMI medium containing 10% FBS and GLP at 37°C and 5% CO2 for 48 h. The cells were pelleted by centrifugation at 500×g at room temperature for 10 min and lysed in RIPA buffer. The extracted proteins were quantified by the Bradford method using a BCA kit according to the supplier’s instructions. 40 μg proteins were subjected to 12% SDS-PAGE and transferred to PVDF membranes. After the membranes were blocked by 5% defatted milk for 1 h at room temperature, they were incubated with anti-cleaved caspase, anti-BAX, and anti-Becl2 antibodies at 4°C overnight and secondary antibodies (goat anti-rabbit or anti-mouse IgG secondary antibodies) at room temperature for 4 h. Enhanced chemiluminescence solution was then drop-added to the membranes and immunoreactive bands were captured with the gel imaging system and analyzed for gray value with the “Image Lab” software.
Table 1. H- and 13C-NMR chemical shifts of GLP5 in D2O at 295K

| Fragments | Glycosyl residues | C1/H1 | C2/H2 | C3/H3 | C4/H4 | C5/H5 | C6/H6 |
|-----------|------------------|-------|-------|-------|-------|-------|-------|
| A         | β-1,6-Glc        | 101.96/4.61 | 72.83/3.22 | 75.75/3.51 | 69.94/3.41 | 71.9/3.79 | 68.98/4.23 |
| B         | β-1,3-Glc        | 102.93/4.78 | 72.75/3.24 | 84.49/3.55 | 69.94/3.21 | 71.2/3.53 | 62.71/3.53 |
| C         | β-T-Glc         | 102.76/4.32 | 71.86/3.32 | 75.62/3.75 | 69.64/3.33 | 71.5/3.52 | 60.71/3.93 |

Figure 1. HPGPC-MALLS analysis of GLP5 on a SB-806HQ Shodex OH pak with a mobile phase of 0.1mol/L Na2SO4 flowing at 0.6 ml/min at 35°C.

Figure 2. Dose-response curve for determining the IC 50 value for GLP5 against Jurkat (black line) and HaCat (red line) cells after exposed to GLP5 for 48 h using the MTT assays. Values represent the mean of at least three independent experiments. ** and *** indicate p<0.01 and p<0.001 compared with the control (one-way ANOVA followed by the Dunnett post-test).
FITC to stain the cells and the results showed that the early apoptotic rate increased from 0.92% in control to 4.35% and 49.22% after exposure to 25 and 50 mg/L GLP5 (Fig. 3), indicating that GLP5 could cause Jurkat cells to die through apoptosis. On the other hand, the apoptotic rate remained barely changed near 1% in HaCat cells after being exposed from 0 to 50 mg/L GLP5 (Fig. 3).

GLP5 upregulates caspase-3 and Bax expression and downregulates Bcl2 expression

We then assessed the expression of caspase-3, a key enzyme involved in apoptosis in Jurkat cells after being exposed to GLP5 using Western blot analysis. The results showed that the expression of cleaved caspase-3 was significantly upregulated after Jurkat cells were exposed to GLP5 in a dose–dose-dependent manner (Fig. 4), indicating that GLP5 could activate caspase-3 and apoptosis signaling pathways. Since the induced apoptosis is likely to change the expression of Bcl2 family proteins, the expressions of Bcl2 (anti-apoptotic) and BAX (pro-apoptotic) after treatment with GLP5 were also assessed using Western blot analysis. Bcl2 expression was reduced significantly after the cells were exposed to 25 mg/L GLP5 after 24 h and was dramatically reduced after the cells were exposed to 50 mg/L GLP5 (Fig. 5). At the same time, BAX expression was significantly increased upon treatment with GLP5 (Fig. 5), particularly upon treatment with 50 mg/L GLP5.

DISCUSSION

Clinically, leukemia is mainly treated with chemotherapy that uses multiple drugs, but the overall prognosis has not been satisfactory due to drug tolerance and serious adverse reactions. Polysaccharides are natural polymers and some of them have been demonstrated to have antitumor activity with less adverse reactions and are considered to be excellent drug candidates in various tumors (Bian et al., 2020; Sadreddini et al., 2017; Wang et al., 2019), although their activity on leukemia is relatively less known. In this study, the cytotoxicity of GLP5, a
polysaccharide extracted from *G. lucidum* was tested on leukemia cells. The results showed that GLP5 has potent cytotoxicity against Jurkat cells with a IC_{50} of 34.5±4.5 mg/L and could activate apoptosis pathways, leading to increased apoptosis and upregulation of pro-apoptotic genes. Therefore, GLP5 may be further investigated as a potential therapeutic agent for leukemia.

A variety of polysaccharides have been found to have inhibitory effect on leukemia cells. Polysaccharide extracted from the fruits of *Lyssium barbarum* was found to be able to enhance the activity of NK cells from leukemia patients against HL-60 cells by upregulating NK-G2D ligand MICA (Xiao & Wu, 2017) and lentinan, a polysaccharide prepared from the shiitake mushroom (*Lentinula edodes*), was shown to inhibit the proliferation of HL-60 cells in a concentration-dependent manner. It also triggered the expression of cleaved PARP, cleaved caspase-3, and cleaved caspase-3 and the release of cytochrome c into the cytoplasm (Ma et al., 2019). In addition, polysaccharides from *Citrusun deserticiola* (Zhang et al., 2016), *Agericus blazei* (Li et al., 2013), and *Pleurotus eryngii* (Chen et al., 2015) also exhibit cytotoxicity against various leukemia lines. Recently, glycosaminoglycan-like polysaccharides extracted from the common cockle (*Ceratodera edule*) have been shown to have antiproliferative activity on chronic myelogenous leukemia and relapsed acute lymphoblastic leukemia cell lines (Aldairi et al., 2018). In this study, GLP5 was found to be a β-(1→3) and β-(1→6) linked glucan based on HPGPC-MALLS and NMR analyses. We used MTT assays to assess the cytotoxicity of GLP5 against the Jurkat cells and found that GLP5 is highly toxic to the Jurkat cells. The IC_{50} is similar to that of lentinan against human lymphocytes (Peter et al., 1988). Subsequent apoptosis assays based on flow cytometry showed that apoptosis was induced after the Jurkat cells were exposed to GLP5, suggesting that at least one of the mechanisms underlying the toxicity is the activation of apoptosis pathways. This is consistent with the results obtained in lentinan on HL-60 cells (Ma et al., 2019). On other hand, when GLP5 was assessed against non-cancerous HaCat cells, no cytotoxicity and apoptosis were observed in the tested concentration ranges, suggesting that GLP5 is selectively toxic to leukemia cells. The reason for this selectivity is unclear. It might be due to the differences between these cells in cell surface ligands/receptors, permissibility to the GLP5 might be due to the differences between these cells in the cells.

Apoptosis, also known as programmed cell death, is one of the most important pathways leading to cell death after cancer is treated with chemotherapy (Johnstone et al., 2002). Induction of defective apoptosis in cancer is one of the major causative approaches for cancer treatment (Goldar et al., 2015; Melet et al., 2008) and apoptosis is considered as a major target for cancer therapy with pro-apoptotic agents (Carneiro & El-Deiry, 2020). To further confirm that GLP5 induced apoptosis in Jurkat cells, we analyzed the expression of several key genes involved in apoptosis pathways and found that pro-apoptotic genes that cleaved caspase-3 and BAX were upregulated and anti-apoptotic gene Bcl-2 was down-regulated after the Jurkat cells were exposed to GLP5, indicating that GLP5 has an impact on apoptosis pathways. These results are consistent with the previous studies with lentinan on HL-60 cells (Ma et al., 2012), *Sargassum fusciforme* polysaccharide on human erythroleukemia cells (Ding et al., 2020) and pectin-like polysaccharide from *Polygala tenuifolia* on pancreatic cancer cells (Bian et al., 2020). Caspase-3 is the best recognized biochemical hallmark of both early and late stages of apoptosis and exists in the form of pro-caspase-3. When apoptosis is induced by a wide variety of apoptotic signals, caspase-3 is hydrolyzed to cleaved caspase-3, resulting in proteolysis and apoptosis (Choudhary et al., 2015; Crowley & Waterhouse, 2016). Therefore, detection of cleaved caspase-3 is considered as a reliable marker for cells that are dying, or that have died by apoptosis (Crowley & Waterhouse, 2016). After incubation with GLP5, the level of cleaved caspase-3 increased as the concentration of GLP5 increases, indicating that GLP5 could induce apoptosis in the Jurkat cells.

In addition, the BCL-2-associated X apoptosis regulator (BAX), is a pro-apoptotic regulator that is involved in a wide variety of cellular activities. This protein increases the opening of the mitochondrial voltage-dependent anion channel (VDAC), leading to the loss of membrane integrity, the release of cytochrome c, and cell death (Kuwana et al., 2020; Maes et al., 2019). Apoptosis induced by various anticancer agents often results in upregulated BAX and downregulated Bcl2 expressions. For example, after being treated with mangiferin, BAX and Bcl2 levels were changed, leading to apoptosis in the CNE2 nasopharyngeal carcinoma cells (Pan et al., 2014), and upregulation of BAX and downregulation of Bcl2 also result in apoptosis induced in various human cancer cell lines by 3-NC (2-amino-4-(3-nitrophenyl)-3-cyano-7-(dimethylamino)-4H-chromene) (Nasri et al., 2015). Altered ratio of proapoptotic and anti-apoptotic Bcl2 family members Bax and Bcl2 after exposure to GLP5 is likely a key driver leading to apoptosis. However, how GLP5 induces the changes of expression of these apoptosis-related genes needs to be further elucidated. In human colon cancer, polysaccharide hydrolysate derived from *G. lucidum* could induce apoptosis to suppress the growth of cells by upregulating Bax, phospho-extracellular regulated protein kinases (P-ERK), and cleaved caspase-3 and downregulating Bcl2, phospho-serine/threonine kinase 1 (p-Akt1), and cyclo-oxygenase-2 (COX-2) (Bai et al., 2020). On other hand, polysaccharide extracted from *G. lucidum* may enhance the activity of NK cells to activate DNAX-associated protein 10/phosphoinositide 3-kinase/extracellular regulated protein kinases to mediate its cytotoxicity (Yang et al., 2019). It can also bind with NK cells to act on several immune receptors including Dectin-1, complement receptor (CR3), and TLR-2/6, then trigger both innate and adaptive responses and enhance opsonic and non-opsonic phagocytosis to exert antitumor activity (Xiao et al., 2020).

Although GLP5 is demonstrated to have potent inhibitory activity against the Jurkat cells and to be a single β-glucan, further studies with more cancer and non-cancerous cell lines are needed to define its in vitro activity and selectivity against cancer. It may be further purified to avoid activity from other compounds to better define its potency. Physical and chemical modifications such as phosphorylation (Hu et al., 2020), microwave irradiation (el Knidri et al., 2018), acetylation, and carboxymethylation (Ma et al., 2012) may be used to generate more potent derivatives with increased solubility and stability for screening drug candidates from GLP5.

**CONCLUSION**

Our experimental data indicate that GLP5 is a β-(1→3) and β-(1→6) linked glucan, it induces apoptosis and inhibits the proliferation of Jurkat cells but not of HaCat cells. The activity is likely mediated through the upregulation of pro-apoptotic genes and downregulation of anti-apoptotic genes.
of anti-apoptotic genes. Thus, this new polysaccharide and its derivatives may be further assessed for selectivity and safety against leukemic cells and normal cells for potential therapeutic use in leukemia and other cancers.

Conflict of interest
None.

REFERENCES

Aldair AF, Ogundipe OD, Pre DA (2018) Antiproliferative activity of glycosaminoglycans-like polysaccharides derived from marine molucules. Mar Drugs 16: https://doi.org/10.3390/md16020063

Amini E, Baharara J, Afzali M, Niekold N (2019) The p53 modulated cytotoxicity of ophiocloa scopodendria polysaccharide against resistance ovarian cancer cells. Anticancer Res 39: 208–214

Bai JH, Xu J, Zhao J, Zhang R (2020) Ganoderma lucidum polysaccharides enzymatic hydrolysis suppresses the growth of human colon cancer cells via inducing apoptosis. Cell Transplant 29: 102062. https://doi.org/10.1080/17474086.2019.1627869

Bewersdorf JP, Shallis RM, Wang R, Huntington SF, Perreault S, Munteanu R, Jurj A, Zollinger X, Zeidan AM (2019) Healthcare expenses for treatment of acute myeloid leukemia. Expert Rev Hematol 12: 611–650. https://doi.org/10.1080/17474086.2019.1627869

Bian Y, Zeng H, Tao H, Huang L, Du Z, Wang J, Ding K (2020) A pectin-like polysaccharide from Pogostemon tilius inhibits pancreatic cancer cell growth in vitro and in vivo by inducing apoptosis and suppressing autophagy. Int J Biol Macromol 162: 107–115. https://doi.org/10.1016/j.ijbiomac.2019.06.054

Boudny M, Tribusek M (2020) ATR-CHK1 pathway as a therapeutic target for acute and chronic leukemia. Cancer Treat Rev 88: 102026. https://doi.org/10.1016/j.cancertr.2020.102026

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2017) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394–424. https://doi.org/10.3322/caac.21492

Carneiro BA, El-Deiry WS (2020) Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol 17: 395–417. https://doi.org/10.1038/s41571-019-0341-y

Chen L, Hao C, Liang T (2017) Studies on inhibition effect on KS62 cells of chemical modified polysaccharide of Phellinus lunsuri. J Shengan Normal U 42: 74–78. https://doi.org/10.15983/j.cnki.jsnmu.2017.02.226

Choudhary GS, Al-Harbi S, Almasan A (2015) Caspase-3 activation is a critical determinant of genotoxic stress-induced apoptosis. Methods Mol Biol 1219: 1–9. https://doi.org/10.1007/978-1-4939-1661-0_1

Crowley LC, Waterhouse NJ (2016) Detecting cleaved caspase-3 in apoptotic cells by flow cytometry. Cold Spring Harb Protoc 2016: https://doi.org/10.1101/pdb.prot87312

Deak D, Goreeza-Andronic N, Sas V, Teodorescu P, Constantinescu C, Blihara S, Paeselloiu C, Zirnea T, Tuicae A, Zitara AA, Galdean S, Steinheber J, Rus I, Rauch S, Richlitzki C, Munteanu R, Jurj A, Zollinger X, Zeidan AM, Novakovic N, Gavric NS, Bozin BN (2016) Ganoderma: insights into anticancer effects. Eisei J Cancer Prev 25: 462–471. https://doi.org/10.1097/CEJ.0000000000000204

Ma L, Chen H, Zang Y, Wang D, Fu L (2012) Chemical modification and antioxidant activities of polysaccharide from mushroom Inonotus obliquus. Carbohydr Polym 89: 371–378. https://doi.org/10.1016/j.carbpol.2012.03.016

Ma L, Dong R, Wang F (2019) Effect of lentinan on apoptosis and PI3K/AKT signaling pathway in leukemia HL-60 cells in vitro. Chin J Pathophysiol 35: 1069–1074. https://doi.org/10.1016/j.cjph.2019.06.017

Mae MS, Groser IA, Fehlman RL, Schlamp CL, Nichelle RW (2019) Completion of BAX recruitment correlates with mitochondrial fission during apoptosis. Sci Rep 9: 16565. https://doi.org/10.1038/s41598-019-5049-w

Mal A, Rda R, Wunsch Filho V (2013) Infection and childhood leukemia: review of evidence. Rev Saude Publica 47: 1172–1185. https://doi.org/10.1590/0034-8410.2013074087543

Melnik A, Song K, B ucz G, Ogas Z, Grzegorzan AR, Khsoravi- Far R (2008) Apoptotic pathways in tumor progression and therapy. Adv Exp Med Biol 615: 47–79. https://doi.org/10.1007/978-1-4020-6554-3_3

Murphy T, Yee KW (2017) Cytarabine and daunorubicin for the treatment of acute myeloid leukemia. Expert Opin Pharmacother 18: 1765–1780. https://doi.org/10.1080/14656566.2017.1391216

Nasert MAH, Mahdavi M, Davoodii J, Tackaloo SH, Goudarzvand M, Neishabouri SH (2015) up regulation of Bax and down regulation of Bel2 during 3NC mediated apoptosis in human cancer cells. Cancer Cell Int 15: 55. https://doi.org/10.1186/s12935-015-0204-2

Pan L, Wang AY, Huang YQ, Luo Y, Ling M (2014) Mangiferin induces apoptosis by regulating Bcl-2 and Bax expression in the CNE2 nasopharyngeal carcinoma cell line. Asian Pac J Cancer Prev 15: 7065–7068. https://doi.org/10.3734/apicp.2014.17.7065

Peter G, Karoly V, Imre B, Janos F, Kaneko Y (1988) Effects of lentinan on cytokines released from activated macrophages and T lympho cytes. Immunopharmacol Immunotoxicol 10: 157–163. https://doi.org/10.3109/08923978809014330

Ranta S, Palomaki M, Levintsen M, Taskiminen M, Halmo A, Savolainen S, Heinlaan M, Harila-Saari A, Nordic Society of Pediatric H, Oncology (2017) Presenting features and imaging of childhood acute myeloid leukemia with central nervous system involvement. Pediatr Blood Cancer 64: https://doi.org/10.1002/pbc.26549

Sadreddini S, Safantaralezh R, Baradaran B, Aghebati-Maleki I, Hosseinpoor-Feizi MA, Shanefiand B, Jafidi-Niafar F, Sadreddini S, Kaffi HS, Younesi Y, Yousefi M (2017) Chitosan nanoparticles as a dual drug/siRNA delivery system for treatment of colorectal cancer. Immunol Lett 181: 79–86. https://doi.org/10.1016/j.imlet.2016.11.013

Sanodiya BS, Thakur GS, Baghel RK, Prasad GB, Bisen PS (2009) Ganoderma lucidum: a potent pharmacological macrofungus. Curr Pharm Bio tech 10: 717–742. https://doi.org/10.2174/1389201097899787577

Sobretedo D, Huang S, Zhang GM (2018) Ganoderma lucidum Polysaccharides as An Anti-cancer Agent. Anticancer Agents Med Chem 18: 667–674. https://doi.org/10.2174/1871528516666171131212466

Takami A (2018) Hematopoietic cell transplantation for acute myeloid leukemia. Int J Hematol 107: 513–518. https://doi.org/10.1007/s12185-018-2412-8

Wang J, Liu G, Ma W, Lu Z, Sun C (2019) Marine bacterial polysaccharide EPS11 inhibits cancer cell growth and metastasis via blocking cell adhesion and attenuating filumform structure formation. Mar Drugs 17: https://doi.org/10.3390/md17010050

Xia J, Yu H, Hua H, Liu J, Xu J, Tang X, Chou G, Wang Z, Mei Q, Liu L (2013) Oligosaccharide from apple induces apoptosis and cell cycle arrest in HT29 human colon cancer cells. Int J Biol Macromol 57: 245–254. https://doi.org/10.1016/j.ijbiomac.2015.03.034

Xia J, Zhao S, Jing Y, Wang J, Liu J, Guo S, Wang Z, Mei Q, Liu L (2013) Oligosaccharide from apple induces apoptosis and cell cycle arrest in HT29 human colon cancer cells. Int J Biol Macromol 57: 245–254. https://doi.org/10.1016/j.ijbiomac.2015.03.034

Yang X, Zhan R, Yao J, Xi C, Du S (2019) Ganoderma lucidum polysaccharide enhanced the antimtumor effects of 5-fluorouracil against
gastric cancer through its upregulation of NKG2D/MICA. *Int J Polymer Sci* 2019: 1–7. https://doi.org/10.1155/2019/4564213

Zeng B, Wang G, Zeng YL (2000a) Effect of spirulina patensis polysaccharide on the activities of lak cell of peripheral blood mononuclear cell in patients with leukemia. *Chin J Mar Drug* 19: 39–41

Zeng BH, GM W, Zeng YL (2000b) Study on effect of spirulina patensis polysaccharide on nk cells from acute leukemia patient in vitro. *Chin J Mar Drug* 19: 45–47

Zhang T, Xu W, Ren K (2016) Comparison of the effect of cistanche deserticola polysaccharide and echinacoside on growth inhibition and cell cycle of K562. *Chin Health Standard Manage* 7: 141–143. https://doi.org/10.3969/j.issn.1674-9316.2016.18.093

Zhao L, Dong Y, Chen G, Hua Q (2010) Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*. *Carbohydrate Polymers* 80: 783–789