Lappaol F, a Novel Anticancer Agent Isolated from Plant Arctium Lappa L.

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Abstract

In an effort to search for new cancer-fighting therapeutics, we identified a novel anticancer constituent, Lappaol F, from plant Arctium Lappa L. Lappaol F suppressed cancer cell growth in a time- and dose-dependent manner in human cancer cell lines of various tissue types. We found that Lappaol F induced G1 and G2 cell-cycle arrest, which was associated with strong induction of p21 and p27 and reduction of cyclin B1 and cyclin-dependent kinase 1 (CDK1). Depletion of p21 via genetic knockout or short hairpin RNA (shRNA) approaches significantly abrogated Lappaol F–mediated G2 arrest and CDK1 and cyclin B1 suppression. These results suggest that p21 plays a crucial role in Lappaol F–mediated regulation of CDK1 and cyclin B1 and G2 arrest. Lappaol F–mediated p21 induction was found to occur at the mRNA level and involved p21 promoter activation. Lappaol F was also found to induce cell death in several cancer cell lines and to activate caspases. In contrast with its strong growth inhibitory effects on tumor cells, Lappaol F had minimal cytotoxic effects on nontumorigenic epithelial cells tested. Importantly, our data also demonstrate that Lappaol F exhibited strong growth inhibition of xenograft tumors in nude mice. Lappaol F was well tolerated in treated animals without significant toxicity. Taken together, our results, for the first time, demonstrate that Lappaol F exhibits antitumor activity in vitro and in vivo and has strong potential to be developed as an anticancer therapeutic. Mol Cancer Ther; 13(1); 49–59. ©2013 AACR.
components isolated from *Arctium Lappa* L., was previously shown to have antitumor effect against pancreatic tumors *in vitro* and in nude mice (12). Arctigenin has a distinct chemical structure from Lappaol F, and it was shown to preferentially kill the nutrition-deprived cancer cells and regulate insulin/insulin-like growth factor-I-mediated Akt signaling (12). Previous studies have shown that under certain circumstances, multiple agents each with its own anticancer properties can be isolated from the same plant, for example, vincristine and vinblastine each isolated from *C. roseus* (6). To identify antitumor constituents from *Arctium Lappa* L., we performed chromatographic isolation using seeds of the plant and acquired more than thirteen lignanoid compounds. Their chemical structures were identified on the basis of the spectroscopic data [UV, MS (mass spectrometry), and NMR (nuclear magnetic resonance)]. Cytotoxicity analysis was performed to determine the antitumor potential of the purified compounds. As reported here, our studies revealed that one of the isolated constituents, named Lappaol F, exhibited strong growth inhibition against various tumor cell lines in cell culture, and it also significantly inhibited HeLa cells growth as a xenograft in nude mice. Mechanistic studies gaining insight into the action of Lappaol F indicate that Lappaol F effectively arrested cell cycle at G1 and G2 phases and induced apoptosis. Lappaol F also altered the function and expression of a number of key regulators of cell cycle. Thus, our studies, for the first time, indicate that Lappaol F could be developed as an anticancer therapeutic.

Materials and Methods

**Cell culture conditions and reagents**

Human cancer cell lines MCF-7, MDA-MB-231, MDA-MB-468, HS578T (breast), RKO and HT29 (colon), A549 (lung, non-small cell), DU145 (prostate), U2OS (osteosarcoma) and A375, SK-Mel-103 (melanoma), RKO p53<sup>+/−</sup>, and p53<sup>−/−</sup> cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FBS (Gemini Bio-Products). K562, HL60, Jurkat (leukemia), HCT116 (colon cancer) cells [p21-proficient (p21<sup>+/−</sup>) or p21-deficient (p21<sup>−/−</sup>)] were grown in RPMI-1640 medium with 10% FBS. HeLa cervical cancer cells were grown in DMEM with 10% FBS. Human nontumorigenic breast epithelial MCF-10A cells were grown in mammary epithelial cell growth medium with supplements provided in the SingleQuots Kit (Lonza).

**Cell line source**

The RKO<sub>p53<sup>−/−</sup></sub> and RKO p53<sup>+/+</sup> cells and HCT116 p21<sup>−/−</sup> and HCT116 p21<sup>+/−</sup> cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). SK-Mel-103 melanoma cell line (originally from Dr. Maria S. Soengas, Melanoma Laboratory, Spanish National Cancer Research Center, Madrid, Spain) were kindly provided by Dr. David M. Markovitz (Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI). The A375 melanoma cell line was kindly provided by Dr. Nihal Ahmad (University of Wisconsin, Madison, WI). The K562 and HL60 human leukemia cells were obtained from Dr. M. Golam Mobi (Upstate Medical University, Syracuse, NY). MCF10A and U2OS cells were purchased from American Type Culture Collection (ATCC). The remaining cell lines used in this study were from the National Cancer Institute, NIH, Bethesda, MD and are also available from the ATCC. These cell lines were not further authenticated in our laboratories.

**Lentivirus-mediated shRNA silencing**

p21 short hairpin RNA (shRNA) constructs were from Open Biosystems. Scramble shRNA construct (Addgene plasmid 1864) was purchased from Addgene. The p21 RNAi targeting sequences used were as follows: p21 RNAi-1: 5′-cgcctaccttctctgctta-3′ and p21 RNAi-2: 5′-ggggatcggactctctgctta-3′. Virus production and infection were performed per protocol provided by Addgene.

**MTT assay**

MTT cell proliferation assays were performed as previously described (13). Briefly, cells seeded in a 12-well plate with or without drug treatment were incubated with 0.5 mg/mL MTT for 1 to 4 hours. The resulting formazan precipitate was dissolved in isopropanol with 0.04 mol/L HCl. Absorbance was read with a Bio-Rad SmartSpec 3100 at 570 nm with background subtraction read at 690 nm.

** Luciferase assays**

Luciferase assays were performed as described in our previous studies (14). Briefly, cells transiently transfected with the p21 promoter luciferase construct (kindly provided by Dr. Bert Vogelstein, Johns Hopkins University) were treated with or without Lappaol F (50 μmol/L). Twenty-four hours later, luciferase activity of each cell lysate was analyzed using the luciferase assay system (Thermo Scientific) with LUMAT LB-9507 luminometer (Berthold Technologies).

**Cell-cycle analysis and mitotic index**

Cell-cycle profile was determined by flow cytometry as we previously described (15). For determining the mitotic index, cells treated with or without Lappaol F were stained with 4′,6-diamidino-2-phenylindole (DAPI). The number of mitotic cells was counted under a fluorescent microscope. More than 600 cells were counted in each sample and experiments were repeated at least three times.

**Western and Northern blot analyses**

Western blotting was done by the standard protocols as we have previously described (15, 16). Sources of the antibodies are as follows: antibodies for p21, CDK2 (cyclin-dependent kinase 2), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were from Santa Cruz Biotechnology; cyclin B1 antibody was from BD Biosciences; and CDK1 and p27 antibodies were from Cell Signaling Technology.
Northern blot analysis was performed as previously described (17) and a full-length p21 cDNA was used as a probe for detecting the expression of p21 mRNA.

**In vivo studies**

All animal studies were approved and performed according to the Animal Care and Use Guidelines of the Animal Ethics Committee at Guangzhou University of Chinese Medicine (Guangzhou, China; document no. syxk (Yue) 2008–0001). HeLa cells used in vivo studies were kindly provided by Prof. David W.F. Fong (Hong Kong Baptist University, Hong Kong). Cells were grown in regular DMEM supplemented with 10% FBS. BALB/c nude mice (female, 4–5-week-old) were first subcutaneously injected with $5 \times 10^6$ of HeLa cells on the back to establish tumor xenografts. Nine days after tumor cell injection, mice with tumor volume of 90 to 290 mm$^3$ were randomized into 3 groups; the average initial tumor volumes in the control and treatment groups were 161.28 mm$^3$ (for mice treated with Lappaol F 5 mg/kg), and 144.5 mm$^3$ (for mice treated with Lappaol F 10 mg/kg), respectively. Mice were then treated with Lappaol F (5 mg/kg/d, $n = 6$) or with equivalent volume of vehicle (5% dimethyl sulfoxide plus 5% Tween 80 in 5% glucose solution, 5 mL/kg/d, $n = 7$) by intravenous injection for 15 days. Tumor size was monitored by measuring two perpendicular diameters with a caliper every 4 days. The tumor volume was calculated as volume $= \text{length} \times \text{width}^2 \times 0.5$. Cases of death and body weight in mice were monitored daily. The animal experiments were terminated on day 15 (tumor size exceeded a mean diameter of 20 mm in the control animals) by sacrificing mice according to the guidelines. Tumor xenografts were then stripped and weighed. All results were expressed as mean $\pm$ SEM. Effects of various treatments were analyzed using the one-way ANOVA analysis and $P$ values $< 0.05$ were considered statistically significant.

**Results**

**Extraction and structural characterization of Lappaol F isolated from Arctium Lappa L.**

The air-dried and powdered seeds of *Arctium Lappa* L. were extracted with methanol (MeOH, 80%) at room temperature. The methanol extract was obtained by removal of methanol in vacuum. The syrup methanol extract was further extracted by petroleum ether, chloroform (CHCl$_3$), and ethyl acetate, respectively. The CHCl$_3$ extract (100 g) was then chromatographed repeatedly on silica gels and Octadecylsilyl silica gel (ODS) columns, eluted with CHCl$_3$/MeOH (99:1–90:10) and MeOH/H$_2$O (30:70–60:40); after these steps of extraction and purification, a colorless amorphous powder compound, named AL12, was obtained together with twelve other compounds. For structural identification, spectroscopic data for all isolated compounds were measured; UV absorption spectra were run on a TU-1901 UV spectrometer (Perkinje General); Electrospray ionization MS (ESI–MS) spectra were measured on an API 2000 liquid chromatography/tandem mass spectrometry apparatus or a MAT95XP mass spectrometer; $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker DRX-400 instrument using tetramethylsilane as an internal standard. The chemical structure of AL12 was identified as Lappaol F, based on information collected, including data from ESI–MS $m/z$ 749 ([M+Cl]$^-$), UV (MeOH) $\lambda_{\text{max}}$ (log$\epsilon$): 232 (4.28), 282 (4.00) nm, and $^1$H- and $^{13}$C-NMR (Supplementary Figs. S1 and S5), matched with those have previously reported (18, 19). The purity of Lappaol F used in this study was assayed as 99.19% by high-performance liquid chromatography (Supplementary Fig. S5). The chemical structure of Lappaol F is shown in Fig. 1A.

**Lappaol F exhibits growth suppression in various tumor cell lines**

Through the initial cytotoxic screening assays, we found that Lappaol F exhibited strong growth inhibitory effect against cancer cell lines representing different tissue types such as colon (HT29, RKO, and HCT116), breast (MCF-7, MDA-MB-231, and MDA-MB-468), cervix (HeLa; Fig. 1B and C). Lappaol F also exhibited growth inhibition of various tumor cell lines of other tissue types such as lung (A549), prostate cancer (DU145), cervix (K562, HL60, and Jurkat), osteosarcoma (U2OS), and melanoma (A375, SK-Mel-103; Supplementary Fig. S6). Lappaol F-mediated cell growth suppression was time- and dose-dependent (Fig. 1D and E) and the estimated absolute EC$_{50}$ was 13.3, 16.8, and 25.2 $\mu$mol/L for MCF-7, MDA-MB-231, and RKO cells, respectively. Interestingly, we also found that Lappaol F exhibited minimal cytotoxicity toward MCF10A noncancerous breast epithelial cells when treated under similar condition (3 days; Fig. 1F) or with prolonged treatment to 6 days (Fig. 1G).

**Lappaol F induces G$_1$ and G$_2$ arrest and cell death**

We sought to determine the mechanism of action via which Lappaol F mediated cell growth suppression. Figure 2A shows the results of flow cytometry analysis of Lappaol F–treated and -untreated cells. The results indicated that Lappaol F significantly increased the 2N G$_1$-phase population or the 4N G$_2$- (or M)-phase population in cells (Fig. 2A). It seems that there is some variation in the Lappaol F–mediated response. For example, MCF-7 and HeLa cells were predominantly arrested in 2N G$_1$ phase, whereas MDA-MB-231 and RKO cells were mainly arrested in 4N G$_2$ (or M) phase (Fig. 2A). Lappaol F also induced cell death in a subset of cell population of some cell lines such as HeLa, MDA-MB-231, and RKO cells. Figure 2A shows that significant increase (from 1%–15%) in the sub-2N cell death population (sub-G$_1$) was also observed in Lappaol F–treated HeLa cells. We also noted that as Lappaol F treatment time prolonged to 96 hours the dead fraction of cell population was further increased (data not shown). To identify whether the 4N populations were arrested in...
G2 or mitotic phase following Lappaol F treatment, mitotic index was determined. Figure 2B shows that in cells treated with the vehicle (DMSO), the mitotic nuclei represented about 3% to 5% (left, arrows), whereas in the Lappaol F–treated cell populations, no mitotic nuclei were observed (Fig. 2B, right). These results were reproducible and indicated that the increased 4N populations observed in Lappaol F–treated RKO and MDA-MB-231 cells represent G2 rather than mitotic arrested cells. Furthermore, cell nuclei in Lappaol F–treated cells were bigger as compared with those in the vehicle-treated cells; apoptotic fragmented nuclei were also noted in Lappaol F–treated cells (Fig. 2B, right, arrows). These results suggest that Lappaol F–induced cell-cycle arrest occurs at both G1 and G2 phases and it also triggers cell death in a subset of tumor cells.

Lappaol F modulates key cell-cycle regulators relevant to the G1 and G2 phases

We investigated the molecular mechanisms via which Lappaol F induces cell-cycle arrest. Figure 3A–D show that the expression levels of CDK inhibitors p21 and p27 were strikingly elevated in Lappaol
F–treated cells, whereas the levels of CDK2, cyclin B1, and CDK1 were clearly reduced in different cell lines. It is well established that CDK2 activity is crucial for G1–S transition, whereas p21 and p27 induction prevents G1–S transition. On the other hand, cyclin B1/CDK1 activities are required for G2–M transition and cyclin B1/CDK1 are needed for the early onset of mitosis (20). Our results thus indicate that Lappaol F modulates a

Figure 2. Lappaol F induces G1 and G2 cell-cycle arrest. A, flow cytometry cell-cycle analyses performed on cells treated with vehicle (DMSO) or Lappaol F (50 μmol/L) for 72 hours. B, DAPI staining of RKO cells treated with Lappaol F (50 μmol/L) for 72 hours. The number of mitotic cells over the total cells (mitotic index, MI) is also shown.
number of cell-cycle regulatory proteins that are essential for cell-cycle progression.

**Effect of Lappaol F on apoptotic signaling**

The aforementioned results indicate that Lappaol F not only induced G1 and G2 cell-cycle arrest but also triggered cell death in a subset of tumor cell population. Next, we sought to examine the effect of Lappaol F on apoptotic signaling. We found that Lappaol F treatment led to caspases-9 and -3 activation in HeLa cells (Fig. 3E). In MCF-7 cells, however, clear evidence of caspase activation was not noted for caspases-8 and -9. As expected, caspase-3 was not detected in MCF-7 cells due to the gene deletion in the exon 3 (21). In Lappaol F–treated MDA-MB-231 cells, activation of caspases-8, -9, and -3 was observed (Fig. 3E). Thus, these results provide biochemical evidence
indicating that Lappaol F activates apoptotic signaling in some cell types.

**p21 is crucial for Lappaol F–mediated cyclin B1/CDK1 downregulation and G2 arrest**

It is well established that p21, as a CDK inhibitor, plays an essential role in the regulation of G1–S transition (22, 23). However, the role of p21 in the G2–M transition is an understudied area. Our aforementioned results indicate that p21 induction occurs concurrently with reduction in cyclin B1 and CDK1, and G2 arrest in Lappaol F–treated RKO and MDA-MB-231 cells (Fig. 3). Previous studies have shown that p21 induction was required for cyclin B1 downregulation mediated by histone deacetylase inhibitor butyrate (24), and p21 was also needed for T-cadherin–mediated G2 arrest (25). We, therefore, sought to determine whether p21 induction plays a role in Lappaol F–mediated CDK1/cyclin B1 suppression and G2 arrest. In this context, we first used the p21-knockout cells to investigate whether loss of p21 affects cyclin B1 and CDK1 expression levels in Lappaol F–treated cells. Figure 4A shows that cyclin B1 and CDK1 reduction caused by Lappaol F was significantly abolished in p21-deficient (p21−/−) cells (lane 4). We further used the lentivirus-mediated RNAi knockdown approach to confirm the role of p21. Figure 4B shows that p21 induction was required for cyclin B1 downregulation mediated by Lappaol F (lane 6).

[Diagram showing expression levels of p21, Cyclin B1, and CDK1 in different conditions.]
approach to study the effect of p21 knockdown on cyclin B1 and CDK1 regulation. As shown in Fig. 4B, cyclin B1 and CDK1 reduction observed in scramble RNAi cells (lane 2) following Lappaol F treatment was diminished in p21 knockdown cells (lanes 4 and 6). We further investigated whether p21 depletion had an effect on Lappaol F–mediated G2 arrest in RKO and MDA-MB-231 cells because Lappaol F predominantly triggered G2 arrest in these cells. As shown in Fig. 4C and D, depletion of p21 by two different shRNAs targeting different regions of p21 transcript significantly reduced the proportion of cells arrested in the G2 phase. These results suggest that p21 is crucial for Lappaol F–mediated cyclin B1/CDK1 suppression and G2 arrest.

Lappaol F upregulation of p21 occurs at the transcriptional level in a p53-independent fashion

We next investigated whether p21 upregulation by Lappaol F occurs at the transcriptional or the posttranscriptional level. Figure 5 shows that the levels of p21 mRNA (Fig. 5A) and protein (Fig. 5B) were both elevated in Lappaol F–treated cells. These results indicate that Lappaol F–mediated p21 upregulation occurs at the transcriptional level although additional regulation occurring at the posttranscriptional level cannot be ruled out. We then determined whether increased level of p21 mRNA by Lappaol F occurred because of induced activity at the p21 promoter. Cells introduced with the p21 promoter luciferase construct were examined for luciferase activity after
being treated or untreated with Lappaol F. As seen in Fig. 5C, p21 promoter activity was significantly enhanced in four different cell lines (RKO, MCF-7, HeLa, and MDA-MB-231) following Lappaol F treatment. It is of note that although RKO and MCF-7 cells harbor the wild-type p53, MDA-MB-231 cells possess mutant p53 (26) and HeLa cells contain the human papillomavirus that inactivates p53 (27). We also noted that Lappaol F treatment did not increase p53 level (Supplementary Fig. S7), whereas p21 expression was strongly induced under similar conditions (Fig. 3–Fig. 5). To further determine whether Lappaol F–mediated p21 upregulation is p53-dependent, RKO p53-proficient (p53+/+) and -deficient (p53−/−) cells were used to study the promoter activity of p21. As shown in Fig. 5D, Lappaol F caused a 3.5-fold induction of the p21 promoter in p53+/+ RKO cells. Interestingly, although the basal levels of p21 was significantly lower in untreated p53-deficient (p53−/−) cells as compared with that in untreated p53-proficient (p53+/+) cells, Lappaol F was able to upregulate the p21 promoter activity by 8.3-fold in the p53−/− cells (Fig. 5D). Although possibility remains that p53 may contribute to p21 regulation in the p53-proficient cells, our data indicate that Lappaol F–mediated p21 transcriptional induction seems to occur in a p53-independent manner.

**Lappaol F suppresses tumor growth in animals**

We also investigated the effect of Lappaol F on nude mice bearing the xenografted tumors. Mice were first injected subcutaneously with HeLa cervical cancer cells to establish tumors. Nine days after tumor cell inoculations, mice were injected intravenously with vehicle or Lappaol F (5 or 10 mg/kg) once daily for 15 days. Our results shown in Fig. 6 revealed a significant inhibition of tumor growth in mice subjected to Lappaol F treatment. As shown in Fig. 6A, after 15 days of drug treatments, Lappaol F inhibited tumor growth by 54% (5 mg/kg/d, n = 7; P < 0.001) and 64% (10 mg/kg/d, n = 6; P < 0.001) relative to the vehicle-treated cohorts (n = 7). Figure 6 also shows that increasing the Lappaol F dose from 5 to 10 mg/kg/d further decreased tumor volume and weight. Importantly, we did not observe lethality or weight loss in mice that were given Lappaol F (5 and 10 mg/kg/d) spanning 15 days of treatment (Fig. 6D). These results indicate that the Lappaol F given to mice was well tolerated and inhibited tumor growth in vivo.
Discussion

In this study, we have identified a novel plant-derived antitumor agent Lappaol F and uncovered its tumor inhibitory potential using cancer cell lines and animal model. Our results indicate that in cell culture, Lappaol F exerts a strong growth inhibition on various tumor cell lines representing different tissues, including colon, breast, lung, cervix, prostate, and melanoma, osteosarcoma, and leukemia. Our studies further indicate that Lappaol F mediates its growth suppression predominantly through inducing G1 and G2 cell-cycle arrest, and in addition to its effect on cell-cycle regulation, Lappaol F also triggers cell death in some tumor cell lines. It is interesting to note that the cell-cycle profiles vary in different cell lines responding to Lappaol F treatment; for instance, MCF-7 cells was predominantly arrested at the G1 phase, whereas RKO and MDA-MB-231 cells mainly arrested at the G2 phase. The variation in response to Lappaol F by these cells could be due to differences in their intrinsic changes that may have occurred during cancer development and/or progression. Studies into the mechanism of action of Lappaol F demonstrate that Lappaol F modulated the expression of a number of key cell-cycle regulators such as p21, p27, cyclin B1 and CDK1, and CDK2 (Fig. 3). It is conceivable that Lappaol F–induced strong induction of p21 and p27 and suppression of CDK2 are sufficient to prevent cell-cycle progression from G1–S, whereas diminished expression of cyclin B1 and CDK1 could cause G2 arrest because the activities of cyclin B1 and CDK1 are required for G2–M transition (20). In this context, our study has further identified that p21 is critical for Lappaol F–mediated cyclin B1 and CDK1 downregulation and G2 arrest. Our results showed that Lappaol F–mediated cyclin B1 and CDK1 reduction was abolished in p21-depleted cells (Fig. 4), and furthermore, Lappaol F–mediated G2 cell-cycle arrest was also significantly altered in the absence of p21 (Fig. 4). Thus, our results suggest that p21 induction is an important event in Lappaol F–mediated cellular responses and it plays a key role in Lappaol F–mediated cyclin B1/CDK1 suppression and G2 cell-cycle arrest.

Our studies also demonstrate that Lappaol F induced strong induction of p21 mRNA expression, which could indicate that the activation of p21 in response to Lappaol F occurs at the transcriptional level. Our data also showed that the enhanced p21 mRNA expression by Lappaol F seemed to occur due to increased p21 promoter activity (Fig. 5). Interestingly, p21 promoter activation following Lappaol F treatment occurred in cells harboring the wild-type p53 (RKO and MCF-7) as well as in cells expressing mutant and nonfunctional p53 (MDA-MB-231 and HeLa). In addition, our data indicate that Lappaol F treatment caused p21 promoter induction in cell lacking the p53 gene (p53−/−) by about 8.3-fold (Fig. 5D). Thus, our results demonstrate that Lappaol F–mediated p21 regulation occurs in a p53-independent manner.

Lappaol F also induced growth suppression in cells expressing wild-type p53 (MCF-7 and RKO) or mutant/nonfunctional p53 (MDA-MB-231 and HeLa). Importantly, our studies also demonstrate that Lappaol F exerted strong growth inhibition on HeLa tumors grafted onto the nude mice (Fig. 6). It is of note that HeLa cells harbor the human papilloma virus that disrupts p53 function (27). Inactivation of p53 is a common feature of human cancer cells and more than half of the human cancers harbor the defective p53 (28). Very encouragingly, we note that Lappaol F given daily at a dose of 5 or 10 mg/kg for 15 days significantly inhibited the growth of xenografted HeLa cells by 54% (P < 0.001) and 64% (P < 0.001) as compared with the vehicle-treated cohorts. In addition, animals seemed to tolerate the treatment of Lappaol F without significant body weight changes during treatment period. Thus, our results, for the first time, indicate that Lappaol F has a tumor suppression function in vitro and in vivo and has a great potential to be developed as an anticancer therapeutic.

At present, there are a number of anticancer drugs in the clinical use that target cell-cycle progression, for example, the vinca alkaloids (vinristine, vinblastine, vinorelbine, and vindesine), the taxanes (paclitaxel or docetaxel), and colchicine (5). These drugs are derived from plants and share a common mechanism of action, which is to induce mitotic arrest. These drugs are considered to achieve this effect by altering microtubule polymerization potential and preventing normal mitotic spindle formation (20). Cancer cells can develop resistance to these chemotherapeutics via a number of mechanisms, such as, for example, alterations in microtubule dynamics including changes in β- and α-tubulin isotype levels or compositions; mutations occurring in tubulins that affect drug binding; protein modifications that modulate tubulin/microtubule dynamic regulatory proteins (reviewed in ref. 29). Such cellular changes are expected to alter the interplay between microtubules and microtubule-targeting drugs, thereby giving rise to drug resistance. Our study has identified that Lappaol F functions to mainly arrest cell-cycle progression at the G1 and G2 phases through regulation of cell-cycle regulatory proteins. The mechanism of action of Lappaol F seems to be different from the above noted microtubule-targeting drugs. It is, therefore, likely that the mechanisms that lead to drug resistance against the above noted microtubule-targeting drugs would not affect the action of Lappaol F. Thus, Lappaol F may be used in cases in which the abovementioned microtubule-targeting drugs have failed.

In summary, our study provides several lines of important evidence demonstrating that Lappaol F has distinct mechanisms of action to suppress cancer cell growth and can also inhibit the growth of p53-defective tumors. Thus, Lappaol F has a strong potential to be developed as a novel anticancer therapeutic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Lappao F as a Novel Antitumor Agent

Authors' Contributions

Conception and design: Q. Sun, M.S. Sheikh, Y. Hu, Y. Huang
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Q. Sun, K. Liu, X. Shen, W. Jin, L. Jiang, Y. Huang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Q. Sun, K. Liu, X. Shen, W. Jin, L. Jiang, Y. Huang

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):

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