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LRD-22, a novel dual dithiocarbamatic acid ester, inhibits Aurora-A kinase and induces apoptosis and cell cycle arrest in HepG2 cells

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ABSTRACT

In this study we investigated the antitumor activity of the novel dual dithiocarbamatic acid ester LRD-22 in vitro and in vivo. Several cancer cell lines were employed to determine the effect of LRD-22 on cell growth, and the MTT assay showed there was a significant decrease in viable tumor cell numbers in the presence of LRD-22, especially in the HepG2 cell line. Colony formation assay also showed LRD-22 strongly inhibits HepG2 cell growth. Evaluation of the mechanism involved showed that inhibitory effects of LRD-22 on cell growth are due to induction of apoptosis and G2/M arrest. LRD-22 inhibited Aurora-A phosphorylation at Thr_{288} and subsequently impaired p53 phosphorylation at Ser_{315} which was associated with the proteasome degradation pathway. Tumor suppressor protein p53 is stabilized by this mechanism and accumulates through inhibition of Aurora-A kinase activity via treatment with LRD-22. In vivo study of HepG2 xenograft in nude mice also shows LRD-22 suppresses tumor growth at a concentration of 5 mg/kg without animals suffering loss of body weight. In conclusion, our results demonstrate LRD-22 acts as an Aurora-A kinase inhibitor to induce apoptosis and inhibit proliferation in HepG2 cells, and should be considered as a promising targeting agent for HCC therapy.

1. Introduction

Liver cancer is the sixth most commonly diagnosed cancer, and the third leading cause of cancer related death in 2008 globally [1]. Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histologic subtype, accounting for 70%–85% of total primary liver cancers [2]. In most cases, HCC is diagnosed predominantly at a more advanced stage in the majority of patients [3]. In late stages cases, the prognosis is generally poor and no effective treatment is available except the molecular targeting agent Sorafenib [3–5]. Identification of novel compounds for HCC therapy is therefore critical.

Dithiocarbamates exhibit important properties in many areas, such as antibacterial or antifungal activity [6, 7], chelation of heavy metals [8], and nitrogen monoxide scavenging [9,10]. Our group developed a simple and convenient one-pot method for synthesis of dithiocarbamates [11]. Several of these compounds were found to have potent antitumor activities [12,13]. The dual dithiocarbamatic acid ester LRD-22, (E)-2-(3-([d][1,3] dioxol-5-yl) acryloyl) propane-1, 3-diyl bis(dimethylcarbamodithioate), is a novel compound with a dual dithiocarbamatic structure.

Aurora-A is a member of a new family of serine/threonine kinases which are essential for controlling normal chromosome segregation and centrosome functions [14]. Activation of AURKA requires binding to specific cofactors including Ajuba, Bora and TPX2, leading to the autophosphorylation of a residue in its T-loop (Thr_{288}) [15]. Its kinase activity increases from late G2-phase onwards and peaks in prometaphase. It is well known that p53 tumor suppressor is a key transcription factor regulating cellular pathways such as cell cycle, apoptosis, DNA repair, angiogenesis and senescence [16]. In response to stress signaling, p53 levels increase to protect cells from tumorigenesis by activating the transcription of downstream target genes which subsequently induce cell G1 or G2/M phase arrest and apoptosis [17]. About 50% of human cancers possess a mutated form of p53 [16,18]. p53 and Aurora-A kinase have therefore been important targets of cancer therapy.

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In this study, we investigated the in vitro and in vivo anti-cancer activity of LRD-22, and found LRD-22 significantly inhibits phosphorylation of Aurora-A kinase T288 and resulting in subsequent induction of G2/M cell cycle arrest and apoptosis by inhibition of p53 degradation. LRD-22 is therefore an Aurora-A inhibitor with promising potential anti-cancer activity, especially against hepatocellular carcinoma.

2. Materials and methods

2.1. Chemicals

The dual dithiocarbamatic acid ester LRD-22 was synthesized in our laboratory. Its structure (Fig. 1A) was confirmed by ^{1}H NMR, ^{13}C NMR (Sup. 1A and 1B). The purity of LRD-22 as measured by high performance liquid chromatography (HPLC) was >99%.

2.2. Reagents and antibodies

DMSO, PBS, polyethylene glycol 400, Tween 80, RNase A were purchased from the Sigma Chemical Company Ltd., MO, USA. PARP, cleaved Caspase-3, phosphoAurora-A and α-Tubulin antibodies were purchased from Cell Signaling Technology Inc., MA,USA. Caspase-3, p53 and β-actin antibodies were purchased from Santa Cruz Biotechnology, Inc., CA, USA. Cyclin B1 and phospho p53 antibodies were purchased from Bioworld Technology, Inc., Shanghai, China. Aurora-A antibody was purchased from Abcam Inc., MA, USA.

2.3. Cell culture

All cell lines were maintained at 37 °C under a 5% CO_2 atmosphere in DMEM medium (Gibco, Life technologies, NY, USA) supplemented with 10% (v/v) FBS (Hyclone laboratories Inc., Victoria, Australia).

2.4. Cell viability assay

Cells were seeded in 96-well plates and incubated for 24 h. After experimental treatment, cells were incubated with 0.5 mg/ml MTT (Sigma Chemical Company Ltd., MO, USA) for 4 h at 37 °C. After discarding supernatant and adding 150 μl DMSO in each well, the 492 nm absorbance was measured using a Sunrise absorbance reader (Tecan Austria GmbH., Salzburg, Austria).

2.5. Colony formation assay

Cells were seeded in 6 cm dishes at a density of 1000 cells/dish and incubated for 24 h. After treatment with indicated concentrations of LRD-22 or DMSO for 48 h, cells were further incubated 2 weeks to allow colony formation. Colonies were then fixed with methanol and stained with Giemsa stain (Sigma Chemical Company Ltd., MO, USA), and colonies with a diameter of more than 1 mm were observed and counted.

2.6. Apoptosis analysis with Hoechst 33342 staining

HepG2 cells were seeded on 24-well culture plates to allow cells to grow as a monolayer. At 40% confluence, cells were treated with 1, 2, 4 or 8 μM of LRD-22 dissolved in DMSO, or DMSO alone for 48 h. After treatment, cells were fixed with ice-cold methanol and acetic acid (v/v: 3/1) at 4 °C for 30 min after washing twice with ice-cold PBS. Cells were later re-washed with ice-cold PBS buffer twice and then stained with 10 μg/ml Hoechst 33342 (Solarbio Science & Technology Company Ltd., Beijing, China) for 3 min. Hoechst 33342 was washed away and changes in cell nuclei were observed and

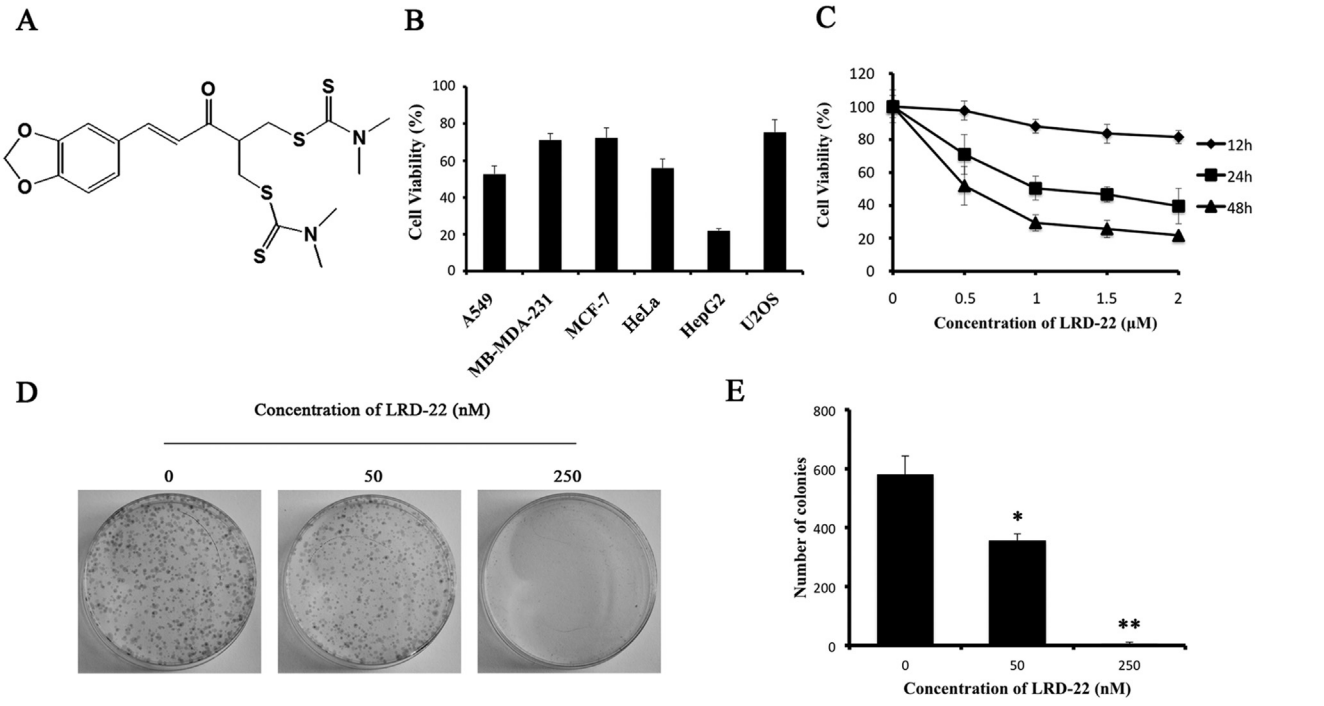

Fig. 1. Inhibitory effects of LRD-22 on cancer cell growth. A. Chemical structure of LRD-22. B. Cell viability after treatment with 2 μM LRD-22 for 48 h in various cancer cell lines. C. Inhibitory effects of LRD-22 on growth in HepG2 cells after treatment with indicated concentrations for 12, 24 or 48 h. D. Colony formation of each group treated with indicated concentrations of LRD-22. E. Statistical results of colony formation assay. Only colonies larger than 1 mm in diameter were counted. Data represent mean ± SD from at least three independent experiments (*P < 0.05, **P < 0.01, difference from control group, as determined by Student's t-test analysis).

captured with fluorescent microscopy (Leica DM500 and Leica AF6000 Modular Systems, Leica Microsystems CMS. GmbH., Wetzlar, Germany).

2.7. Annexin V-FITC/PI staining

After treatment with indicated concentrations of LRD-22 for 24 or 48 h, HepG2 cells were harvested using trypsin without EDTA, and washed with PBS. Cell pellets were then stained with 200 μl binding buffer containing FITC-labeled Annexin V (Invitrogen, Life technologies, NY, USA) for 15 min and PI (Sigma Chemical Company Ltd., MO, USA) for 5 min at room temperature. The samples were then kept on ice and immediately subjected to flow cytometry (BD FACS Calibur, Becton Dickinson, Immunocytometry Systems, CA, USA). Data were collected and analyzed with CellQuest software.

2.8. Cell cycle analysis

Cells were incubated in DMEM containing 0.5% FBS overnight for synchronization, and then treated with indicated concentrations of LRD-22 for 24 h at 37 °C. Cells were collected and fixed in 1 ml of cold 70% ethanol at −20 °C overnight. Cell pellets were then collected by centrifugation, followed by PBS washes. Finally, 0.5 ml of propidium iodide (PI) staining solution (50 μg/ml of PI and 8 μg/ml of DNase-free RNase) was added to the samples, and incubated for 30 min at room temperature. After staining, samples were immediately analyzed using flow cytometry (BD FACS Calibur, Becton Dickinson, Immunocytometry Systems, CA, USA). Data were collected and analyzed with CellQuest software.

2.9. Western blot analysis

After 24 h incubation in 6 cm plates, HepG2 cells were treated with indicated concentrations of LRD-22 for 48 h, then harvested and washed with PBS. Protein was extracted using SDS lysis buffer containing 1% phosphatase inhibitor and 1% protease inhibitor cocktail (Sigma Chemical Company Ltd., MO, USA). Total protein concentrations were determined using the BCA™ Protein Assay kit (Novagen, EMD Chemicals Inc., CA, USA) according to the manufacture's instructions. Equal amounts of protein were subjected to SDS–PAGE, and then electrically transferred onto nitrocellulose membranes. Membranes were subsequently blocked for 1 h with 5% fat free dry milk in TBS-0.1% Tween 20 (TBST), and then incubated with primary antibody overnight at 4 °C. The membrane was then incubated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., USA) for 1 h at room temperature. After repeated washes with TBST, proteins were visualized using an ECL advanced Western blotting detection kit (PekinElmer, Inc., MA, USA) and Kodak film. Densitometric measurements of band intensity in these Western blots were performed using Image J Software (NIH, USA).

2.10. In vivo study

Female BALB/c nude mice (CAnN.Cg-Foxn1nu/CrlVr, 4 weeks of age) were purchased from Vital River Laboratories, Beijing, China, and group housed under conditions with a constant photoperiod (12-h light/12-h dark) and ad libitum access to sterilized food and water. All experimental procedures using mice were carried out in accordance with protocols approved by The Medical Animal Care and Welfare Committee of Peking University. Ectopic tumors were established in nude mice by subcutaneous injection of 1×10^6 HepG2 cells in a total volume of 0.1 ml of physiological saline for each mouse. Tumor establishment and growth was monitored twice weekly by measurement with calipers (tumor volume = width^2 × length × 0.5). Mice with established tumors (mean initial tumor volume 30 mm^3) were randomized into three groups (n = 8) that received every other day intraperitoneal injections of LRD-22 at 5 mg/kg, 10 mg/kg or vehicle (physiologic saline/polyethylene glycol400/DMSO/Tween 80; 70:10:10:10 by volume) for 2 weeks. Body weight and tumor volume were measured twice per week. At the study endpoint, mice were euthanized and tumors were excised, fixed in paraformaldehyde or snap frozen and stored at −80 °C.

2.11. Statistical analysis

All experiments were performed at least three times. Data is expressed as mean ± standard deviation (SD). Significant differences from the controls for each experimental condition were assessed using the Student's t-test, and P values <0.05 were regarded as statistically significant.

3. Results

3.1. LRD-22 inhibits survival and proliferation of HepG2 cells

Based on the one-pot method for the synthesis of dithiocarbamates developed by our laboratory group, we synthesized the compound (E)-2-(3-([d][1, 3] dioxol-5-yl) acryloyl) propane-1, 3-diyl bis (dimethylcarbamodithioate), and designated it LRD-22 (Fig. 1A). The effects of LRD-22 were tested in variant cancer cell lines, including breast cancer cell lines MB-MDA-231 and MCF-7, the lung cancer cell A549, HeLa cervical cancer cells, hepatocellular carcinoma HepG2 cells, and U2OS osteosarcoma cells. To evaluate the inhibitory effects of LRD-22 in these cancer cells, MTT assays were performed. As shown in Fig. 1B, LRD-22 exhibited broad tumor inhibitory activity at a concentration of 2 μM, especially in the HepG2 cell line.

We therefore focused on the anti-cancer activity of LRD-22 in HepG2 cells, and measured its dose- and time-dependent anticancer properties. As shown in Fig. 1C, LRD-22 exhibited obvious inhibitory effects on HepG2 cells in both a dose- and time-dependent manner, with IC_{50} values of about 1 μM in 24 h and 0.5 μM in 48 h.

To further confirm the inhibitory effects of LRD-22 on cell growth, colony formation assays were performed. HepG2 cell colonies treated with LRD-22 were smaller and fewer in number than those of the untreated group (Fig. 1D). As shown in Fig. 1E, HepG2 cells treated with 50 nM LRD-22 formed only 61% of the total number of control group colonies, and cells treated with 250 nM LRD-22 formed only 1% of the number of colonies formed by the control group.

3.2. LRD-22 induces HepG2 cell apoptosis

LRD-22 exhibited obvious tumor inhibitory effect in HepG2 cells, but the specific molecular mechanism involved was unclear. It is well known that apoptosis is important in tumor chemotherapy, and cell apoptosis was therefore evaluated. Morphologic observation of HepG2 cells stained with blue fluorescent DNA dye Hoechst 33342 was performed. As shown in Fig. 2A, cells in the control group had normal cell nuclei morphology whereas cells in LRD-22 treatment groups exhibited typical apoptotic characteristics, including nuclei shrinkage, chromatin condensation and nuclear fragmentation.

Annexin V-FITC/PI stained apoptotic assays were also performed. Flow cytometric assay showed that after incubation with LRD-22 at indicated concentrations for 24 h, the percentage of apoptotic HepG2 cells increased from 6.71% in the control group to

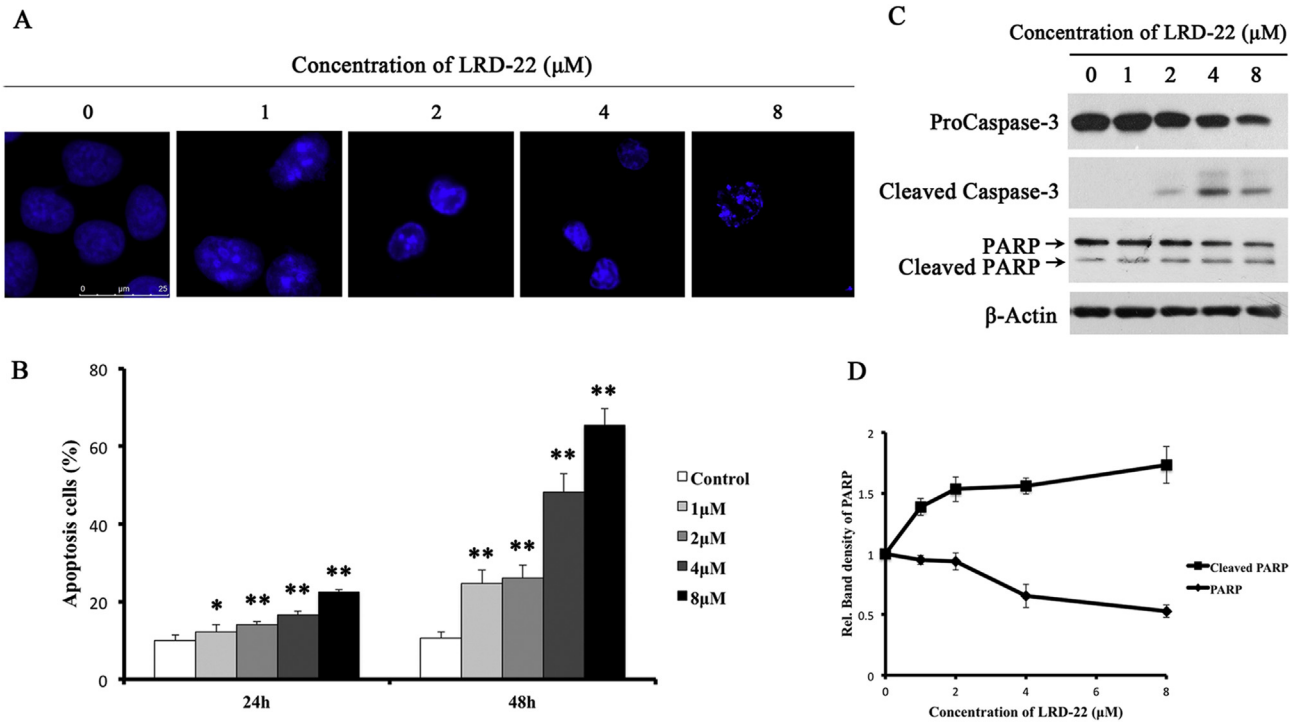

Fig. 2. LRD-22 induced apoptosis in HepG2 cells. A. Cells were stained with Hoechst 33342 after treatment with LRD-22 at indicated concentrations for 48 h and observed under a fluorescence microscope. Morphological changes were observed. B. Representative flow cytometry histograms show percentages of cells in early and late stages of apoptosis. Data represent mean ± SD from at least three independent experiments (*P < 0.05, **P < 0.01, difference from the control groups, as determined by Student's t-test analysis). C. Western blot analysis of Caspase-3 and PARP from protein extracts of HepG2 cells treated with 0 (DMSO control), 1, 2, 4 or 8 μM LRD-22 for 24 h. D. Image J analysis of band densities of PARP and cleaved PARP, as compared with β-Actin. The band density of control groups was considered to be 1.

15.11% in the group treated with 8 μM LRD-22 (Fig. S2). As shown in Fig. 2B, more than 65% cells showed apoptosis after treatment with 8 μM LRD-22 for 48 h. These results demonstrated that LRD-22 induces apoptosis in HepG2 cells in a time- and dose-dependent manner.

Moreover, we examined the involvement of Caspase-3 and PARP in LRD-22 treated cells to investigate the mechanism of the observed apoptotic phenomena. Caspase-3 is considered to be one of the central effectors in the caspase-dependent apoptotic pathway, and it is activated by proteolytic cleavage by zymogen into small subunits [19]. We analyzed the protein level of ProCaspase-3 and cleaved Caspase-3 in HepG2 cells with or without LRD-22 treatment by Western blot analysis. As shown in Fig. 2C, LRD-22 induced a dose-dependent decrease of the caspase-3 35 kDa precursor associated with an increase in 17 kDa cleaved caspase-3. As poly (ADP-ribose) polymerase (PARP) is one of the main cleavage targets of caspase-3 [20], we further investigated the protein levels of PARP and cleaved PARP, and a dose-dependent decrease of 116 kDa PARP with a corresponding increase in 89 kDa cleaved PARP were observed (Fig. 2D). Identification of caspase-3 and PARP cleavage as compared to the control group argue LRD-22 induced HepG2 cell apoptosis is caspase pathway dependent.

3.3. LRD-22 blocks the cell cycle at G2/M

To further explore the effect of LRD-22 on cell proliferation, we evaluated cell cycle arrest to determine whether it contributes to cell growth inhibition by LRD-22. For cell cycle analysis, HepG2 cells were treated with LRD-22 for 24 h and then stained with PI to evaluate their DNA content by FACS. The results showed that the percentage of cells in G2/M phase increased when HepG2 cells were treated with indicated concentrations of LRD-22 (Fig. 3A). As shown in Fig. 3B, cells in G2/M phase increased from 16% in the control group to 35% in the 8 μM treatment group. Moreover, LRD-22 brought about arrest of cells in G2/M phase in a dose-dependent manner.

It is well recognized that Cyclin B is a key regulator which controls G2/M transition and mitosis in all eukaryotes, and destruction of Cyclin B1 is necessary for mitotic exit [21,22]. To further validate G2/M arrest is brought about by LRD-22, we therefore investigated the expression of Cyclin B1in LRD-22 treated HepG2 cells by Western blot analysis. As shown in Fig. 3C and D, expression of Cyclin B1 was increased almost 4 fold in the 8 μM LRD-22 treatment group as compared with the control group. Taken together, our results argue LRD-22 inhibits proliferation of HepG2 cells by G2/M cell cycle arrest.

3.4. LRD-22 induces apoptosis and cell cycle arrest in G2/M through inhibition of aurora-A/p53 pathway

p53 tumor suppressor protein is a multifunctional transcription factor involved in regulation of cell proliferation and apoptosis [23], and p53 levels can be regulated by inhibition of degradation via phosphorylation of p53 at Ser_{315} [24]. To further explore the molecular mechanisms by which LRD-22 induces HepG2 cell apoptosis and G2/M cell cycle arrest, we investigated the effects of LRD-22 on protein level and the p53 phosphorylation. Upon treatment with LRD-22 at variant concentrations, p53 levels increased in a dose-dependent manner as shown in Fig. 4A and 4B. This change in p53 levels corresponded to decreases in phosphorylated p53 at Ser_{315} when LRD-22 was applied. These results

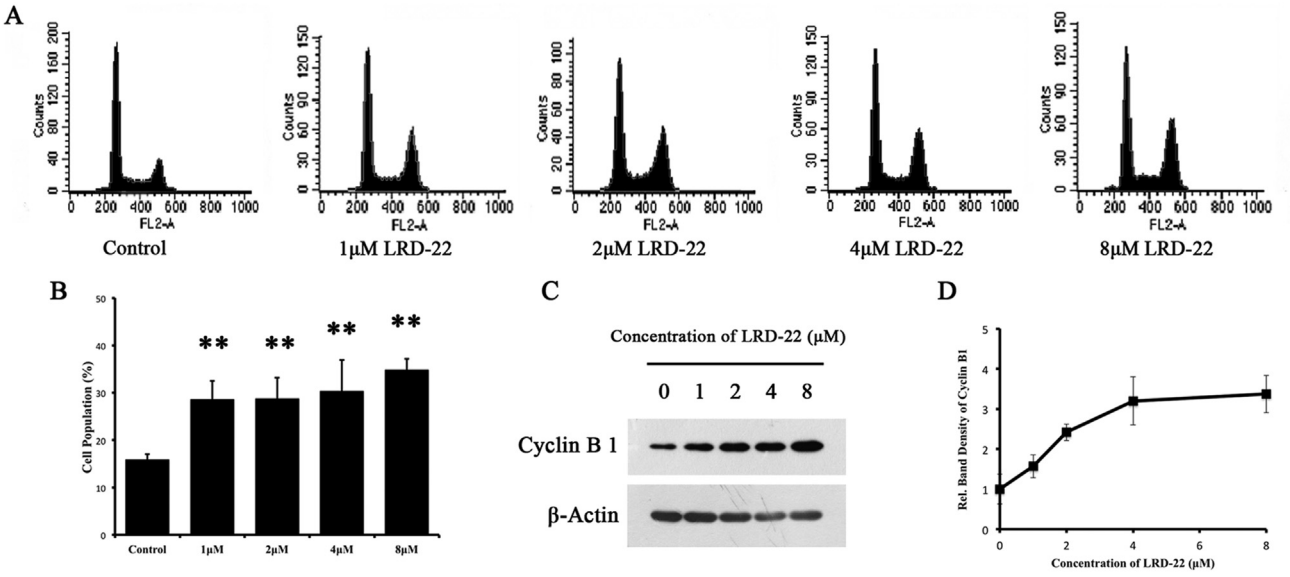

Fig. 3. LRD-22 blocked the cell cycle at G2/M. A. Flow cytometry cell cycle analysis after treatment with LRD-22 at indicated concentrations for 24 h. B. Statistic analysis of the percentages of cells in G2/M phase after treatment with the indicated concentrations of LRD-22 for 24 h. Data represent mean ± SD from at least three independent experiments (*P < 0.05, **P < 0.01, difference from the control group, as determined by Student's t-test analysis). C. Western blot analysis of protein extracts obtained from HepG2 cells treated with 0 (DMSO control) 1, 2, 4 and 8 μM LRD-22 for 24 h. Cyclin B1 was tested and β-actin was used as an internal control. D. Image J analysis of band density for Cyclin B1, as compared to the β-actin group.

demonstrate LRD-22 induces apoptosis and G2/M cell cycle arrest by stabilization of p53 through inhibition of phosphorylation at Ser_{315}.

The mitotic kinase Aurora-A phosphorylates p53 at Ser_{315} in M phase and promotes protein degradation in a MDM2 mediated ubiquitin-proteasome pathway dependent manner resulting in regulation of p53 protein levels [24]. Inhibition of Aurora-A can promote accumulation and activation of p53. Expression of Aurora-A was therefore evaluated by Western blot. A significant decrease in phosphorylated Aurora-A at Thr_{288} was observed in the presence of LRD-22 as shown in Fig. 4C. After treatment with LRD-22 for 24 h, phospho-Aurora-A decreased in a concentration dependent manner, while expression of total Aurora-A showed little change (Fig. 4D).

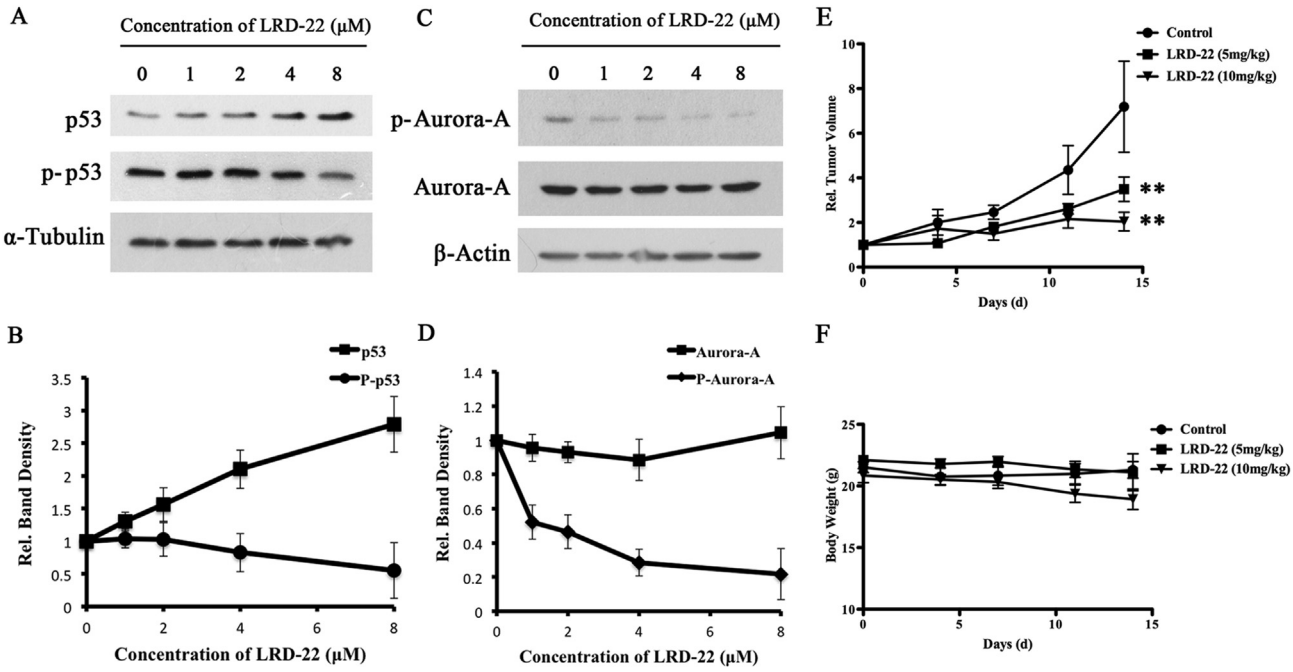

Fig. 4. LRD-22 inhibits Aurora-A phosphorylation in vitro and suppresses HepG2 xenograft tumor growth in vivo. A. Western blot analysis of protein extracts from HepG2 cells treated with 0 (DMSO control), 1, 2, 4 and 8 μM LRD-22 for 24 h p53 and phosphorylated p53 at Ser_{315} were analyzed. α-Tubulin was used as an internal control. B. Image J analysis of band density of p53 and phosphorylated p53 at Ser_{315}, as compared to α-Tubulin. C. Western blot analysis of protein extracts obtained from HepG2 cells treated with 0 (DMSO control), 1, 2, 4 and 8 μM LRD-22 for 24 h. Aurora-A and phosphorylated Aurora-A at Thr_{288} were analyzed and β-actin was used as an internal control. D. Image J analysis of band density of Aurora-A and phosphorylated Aurora-A at Thr_{288}, as compared to β-actin. E. LRD-22 suppressed HepG2 xenograft tumor growth in nude mice. Data was plotted as mean ± SD (n = 8 mice, **P < 0.01, difference from control group, as determined by Student's t-test analysis). F. Body weight measurements over the treatment period.

3.5. LRD-22 suppresses HepG2 xenograft tumor growth in vivo

LRD-22 exhibited significant anti-cancer activity in vitro, especially in HepG2 cells. its in vivo capacity for retarding tumor growth was therefore further assessed in nude mice. Mice bearing subcutaneous xenograft tumors generated from HepG2 cells were randomized into three groups and injected with different concentration of LRD-22 (5 mg/kg, 10 mg/kg) or vehicle every other day. Tumor volume was measured twice a week to evaluate the effects of LRD-22.

Tumor volume measurements revealed that treatment with LRD-22 inhibited HepG2 tumor growth as compared with vehicle treated controls after a treatment period of two weeks as shown in Fig. 4E. Moreover, the inhibitory effect of LRD-22 on xenograft tumor growth was dose dependent. In the 5 mg/kg treatment group, mean tumor growth showed a 3.5 fold increase in two weeks, while the mean tumor volume in the control group showed an increase of 7 times the initial volume. After treatment with 10 mg/kg LRD-22 for 2 weeks, xenograft tumor showed only a two folds increase in volume. These data show LRD-22 suppressed HepG2 xenograft tumor growth in vivo, and this inhibitory effect was dose dependent.

Mouse body weight was also determined twice a week as a basic measure of animal well being and LRD-22 toxicity. Mean body weight in 10 mg/kg LRD-22 treated mice decreased slightly but not significantly over the period of treatment (Fig. 4F), while body weight in the 5 mg/kg LRD-22 treatment group and in the control group was maintained.

4. Discussion

In this study, we determined that LRD22 has broad tumor inhibitory activity at a concentration of 2 μM. LRD-22 exhibited dose- and time-dependent inhibitory effects with an IC_{50} of about 0.5 μM when treated for 48 h, and this effect was particularly evident in HepG2 cells. A HepG2 xenograft tumor model confirmed this LRD-22 tumor inhibitory effect at a concentration of 5 mg/kg with about 50% suppression of tumor growth. Study of the mechanism involved demonstrated LRD-22 significantly induces HepG2 cell apoptosis through the Aurora-A/p53 pathway.

Hiroshi Katayama et al. reported p53 may be phosphorylated at Ser_{315} by Aurora-A kinase, leading to its ubiquitination by MDM2 and proteolysis. Inhibition of Aurora-A kinase activity promotes p53 accumulation, causing upregulation of checkpoint–response pathways and protecting cells from tumorigenesis [24]. As noted above, Aurora-A kinase phosphorylates p53 at Ser_{315} and down regulates its stability, promoting cell transformation. Inhibition of Aurora-A kinase may induce G2/M arrest due to stabilization of p53 [24]. Moreover, Aurora-A kinases are upregulated in several human cancers and are correlated with poor prognosis [25]. These kinases are therefore believed to be important anti-cancer drug targets. A number of small-molecule Aurora kinase inhibitors have been developed over recent years and several compounds are currently in various stages of clinical trials [26].

Based on our results, we propose that LRD-22 inhibits cancer growth by induction of cell cycle arrest and apoptosis through the Aurora-A/p53 pathway. Aurora-A is inhibited by LRD-22, and at the same time p53 is activated to induce cell G2/M arrest and apoptosis. The tumor growth inhibitory activity of LRD-22 was effective not only in vitro but also in vivo.

This is the first time LRD-22, which is a dual dithiocarbamic acid ester, has been reported to be a novel Aurora-A kinase inhibitor with significant anti-cancer effect. LRD-22 is a promising targeting agent for HCC.

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Conflict of interest

All authors have declared they have no conflict of interest for publication of this paper.

Transparency document

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.102.

Appendix A. Supplementary data

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References

[1] F. Bray, A. Jemal, N. Grey, J. Ferlay, D. Forman, Global cancer transitions according to the human development Index (2008–2030): a population-based study, Lancet Oncol. 13 (2012) 790–801.
[2] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer J. Clin. 61 (2011) 69–90.
[3] C. Barone, D. Koeberle, H. Metselaar, G. Parisi, D. Sansonno, G. Spinzi, Multidisciplinary approach for HCC patients: hepatology for the oncologists, Ann. Oncol. 24 (Suppl. 2) (2013) ii15–23.
[4] A. Villanueva, V. Hernandez-Gea, J.M. Llovet, Medical therapies for hepatocellular carcinoma: a critical view of the evidence, Nat. Rev. Gastroenterol. Hepatol. 10 (2013) 34–42.
[5] J.M.L. Alejandro Forner, Bruix Jordi, Hepatocellular carcinoma, Lancet 397 (2012).
[6] H. Imamura, N. Ohtake, H. Jona, A. Shimizu, M. Moriya, H. Sato, Y. Sugimoto, C. Ikeura, H. Kiyonaga, M. Nakano, R. Nagano, S. Abe, K. Yamada, T. Hashizume, H. Morishima, Dicationic dithiocarbamate carbapenems with anti-MRSA activity, Bioorg. Med. Chem. 9 (2001) 1571–1578.
[7] C. Len, Synthesis, Antifungal Activity, Of novel Bis(dithiocarbamate) derivatives of Glycerol, J. Agric. Food Chem. 44 (1996) 2856–2858.
[8] S. Hidaka, T. Funakoshi, H. Shimada, M. Tsuruoka, S. Kojima, Protective effect of n-Benzyl-d-glucamine dithiocarbamate against renal toxicity in rats during repeated cis-diamminedichloroplatinum administrations, Ren. Fail. 17 (1995) 539–550.
[9] C.S. Lai, Conjugates of Dithiocarbamates with Pharmacologically Active Agents and Uses Therefore, United State Patent. (1999) Patent US5916910, 1999-06-29.
[10] A.F. Vanin, X. Liu, A. Samouilov, R.A. Stukan, J.L. Zweier, Redox properties of iron–dithiocarbamates and their nitrosyl derivatives: implications for their use as traps of nitric oxide in biological systems, Biochim.Biophys. Acta (BBA) – General Subj. 1474 (2000) 365–377.
[11] J.L. Cui, Z.M. Ge, T.M. Cheng, R.T. Li, An efficient one-pot synthesis of 2-hydroxyalkyl dithiocarbamates, Synth. Commun. 33 (2003) 1969–1976.
[12] X.L. Hou, Z.M. Ge, T.M. Wang, W. Guo, J.R. Cui, T.M. Cheng, C.S. Lai, R.T. Li, Dithiocarbamic acid esters as anticancer agent. Part 1: 4-substituted-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl esters, Bioorg. Med. Chem. Lett. 16 (2006) 4214–4219.
[13] X.W. Ji, R.T. Li, Z.Q. Li, L. Li, X.Y. Shao, S.Y. Wang, Y. Yuan, T.Y. Zhou, W. Lu, Inhibition of EGFR autophosphorylation plays an important role in the anti-breast cancer efficacy of the dithiocarbamate derivative TM208, Acta Pharmacol. Sin. 35 (2014) 239–247.
[14] D.M. Glover, M.H. Leibowitz, D.A. McLean, H. Parry, Mutations in< i> aurora</ i> prevent centrosome separation leading to the formation of monopolar spindles, Cell 81 (1995) 95–105.
[15] P.J. Scutt, M.L. Chu, D.A. Sloane, M. Cherry, C.R. Bignell, D.H. Williams, P.A. Eyers, Discovery and exploitation of inhibitor-resistant aurora and polo kinase mutants for the analysis of mitotic networks, J. Biol. Chem. 284 (2009) 15880–15893.
[16] A.J. Levine, M. Oren, The first 30 years of p53: growing ever more complex,, Nat. Rev. Cancer 9 (2009) 749–758.
[17] K.H. Vousden, C. Prives, Blinded by the light: the growing complexity of p53, Cell 137 (2009) 413–431.
[18] J. Momand, D. Jung, S. Wilczynski, J. Niland, The MDM2 gene amplification database,, Nucleic Acids Res. 26 (1998) 3453–3459.
[19] M.O. Hengartner, The biochemistry of apoptosis,, Nature 407 (2000) 770–776.

[20] Z. Herceg, Z.-Q. Wang, Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death, Mutat. Res./Fundam. Mol. Mech. Mutagen. 477 (2001) 97–110.
[21] T.A. Chan, P.M. Hwang, H. Hermeking, K.W. Kinzler, B. Vogelstein, Cooperative effects of genes controlling the G2/M checkpoint, Genes Dev. 14 (2000) 1584–1588.
[22] P. Clute, J. Pines, Temporal and spatial control of cyclin B1 destruction in metaphase, Nat. Cell Biol. 1 (1999) 82–87.
[23] A.J. Giaccia, M.B. Kastan, The complexity of p53 modulation: emerging patterns from divergent signals,, Genes Dev. 12 (1998) 2973–2983.
[24] H. Katayama, K. Sasai, H. Kawai, Z.-M. Yuan, J. Bondaruk, F. Suzuki, S. Fujii, R.B. Arlinghaus, B.A. Czerniak, S. Sen, Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53, Nat. Genet. 36 (2004) 55–62.
[25] O. Gautschi, J. Heighway, P.C. Mack, P.R. Purnell, P.N. Lara, D.R. Gandara, Aurora kinases as anticancer drug targets, Clin. Cancer Res. 14 (2008) 1639–1648.
[26] M.R. Green, J.E. Woolery, D. Mahadevan, Update on Aurora kinase targeted therapeutics in oncology, Expert Opin. Drug Discov. 6 (2011) 291–307.