Inorganic Chemistry Communications 67 (2016) 40–43

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Short communication

A cyclometalated iridium(III) complex that inhibits the migration and invasion of MDA-MB-231 cells

Jinquan Wang [a,*], Xiaojuan Hou [b], Zizhuo Zhao [c], Huaben Bo [a], Qizhu Chen [a]

[a] Guangdong Provincial Key Laboratory of Biotechnology Candidate Drug Research, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[b] Huaihua Medical College, Huaihua 418000, PR China
[c] Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, PR China

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 19 February 2016
Accepted 26 February 2016
Available online 3 March 2016

Keywords:
Iridium(III) complex
Invasion
Migration
Antimetastasis
Anticancer

ABSTRACT

A cyclometalated iridium(III) complex, $[Ir(ppy)_2(PCN)]Cl$ (**Ir1**, ppy = 2-phenylpyridine, PCN = 2-(4-cyanophenyl)imidazo[4,5-f] [1,10] phenanthroline), was synthesized and characterized in the present study. **Ir1** inhibited the proliferation, migration and invasion of MDA-MB-231 human breast cancer cells in a dose-dependent manner. Moreover, **Ir1** down-regulated the phosphorylation of AKT/ERK signal pathways. According to confocal fluorescence microscopy analysis, **Ir1** was primarily localized within the mitochondria and induced apoptosis through an intrinsic mitochondria-mediated apoptotic pathway. Thus, **Ir1** exhibited both antimetastatic and antineoplastic properties, indicating that **Ir1** may be a viable drug candidate in antimetastasis and anticancer therapies.

Breast cancer is one of the leading causes of death in women due to its high metastasis rate and ability to invade the lungs, lymph nodes, and bones during the terminal phase of this disease [1]. The mechanism of tumor metastasis involves cell proliferation, migration, and invasion. Several signaling pathways regulate the process of metastasis, including AKT/ERK and other metastasis-related protein kinases [2]. Despite current progress in medicine, metastasis causes 90% of deaths from breast cancer and leads to poor prognosis and clinical outcome. Hence, metastasis remains a major clinical challenge in cancer treatment [3]. Fortunately, research on transition metal-based drugs offers hope in the fight against breast cancer. For instance, two ruthenium(III) compounds, NAMI-A and KP1019, have successfully entered clinical trials due to its ability to combat the development of solid tumor metastasis [4]. Other types of transition metal complexes should be evaluated to determine if they possess similar anticancer properties. In recent years, iridium-based anticancer agents have received significant attention as potential anticancer candidates. These organometallic compounds are attractive prospects for the design of anticancer agents due to their versatile structures, potential redox features, and wide range of ligand substitution patterns [5]. Several reports have shown that iridium-based complexes with antiproliferation properties induce the apoptosis of various tumor cells. For example, fac-[Ir(N^N)(DMSO)Cl_3], a diimine complex designed by Sheldrick, showed potent cytotoxicity toward HT-29× and MCF-7 human cancer cell lines [6]. P. J. Sadler and coworkers synthesized several organometallic complexes and showed that $[Ir(N^N)(\eta^5\text{-}C_5Me_5)Cl]^+$ reacted with 9-ethylguanine, exerting antiproliferative effects on several human cancer cell lines [7]. Z. Liu synthesized **1-py**, an iridium complex that generated H_2O_2 *via* catalytic hydride transfer from coenzyme NADH to oxygen, and showed that the compound was more potent (in nanomolar amounts) than cisplatin toward a wide range of cancer cells [8]. Recently, Z. W. Mao reported that cyclometalated iridium(III) complexes containing bis-N-heterocyclic carbene (NHC) ligands targeted the mitochondria of tumor cells and eradicated cisplatin-resistant cancer cells [9]. However, to the best of our knowledge, only a few studies on the inhibition of tumor metastasis by iridium(III) complexes have been reported. Thus, the aim of the present study was to evaluate the antimetastasis effects of a novel cyclometalated iridium(III) complex (**Ir1**) using a series of *in vitro* bioassays.

The PCN ligand was prepared according to previously reported procedures [10]. The iridium(III) complex was prepared by mixing $[Ir(ppy)_2Cl]_2$ and PCN in a solution of CH_3OH–CH_2Cl_2 (2:1) at reflux for 6 h. (Fig. 1A). The iridium(III) complex was purified by column chromatography and was characterized by elemental analysis, ES-MS, ^1H NMR and electronic spectroscopy (Fig. S1–S3). MDA-MB-231, a highly invasive breast cancer cell line, was chosen as the model cell line. Cell migration is an important step in tumor metastasis [11]; therefore, we performed wound-healing assays to determine the effects of **Ir1** on MDA-MB-231 cell migration. The results showed that the wound size of the control cells decreased after 24 h (Fig. 1B). On the contrary, after being treated with different concentrations (2.5, 5, and 10 μM) of **Ir1**, the wound size of **Ir1**-treated cells was significantly larger than

* Corresponding author.
E-mail address: solo_wjq@126.com (J. Wang).

http://dx.doi.org/10.1016/j.inoche.2016.02.018
1387-7003/

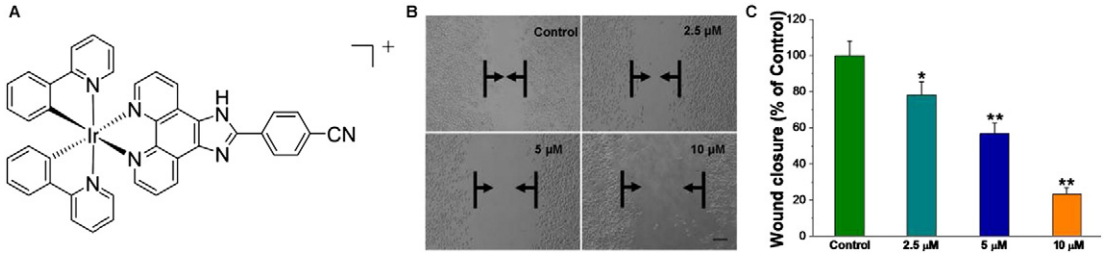

Fig. 1. (A) Chemical structure of **Ir1**. (B) Effects of **Ir1** on MDA-MB-231 cells migration in wound-healing assays. (C) Does-dependent wound closure in MDA-MB-231 cells after incubation with **Ir1** for 24 h. Scale bar, 20 μm. Cell migration was quantified and expressed on 100% (% of control). Data represent by the mean ± SD (bars) of three experiments. * = $p < 0.05$; ** = $p < 0.01$, compared with the control group.

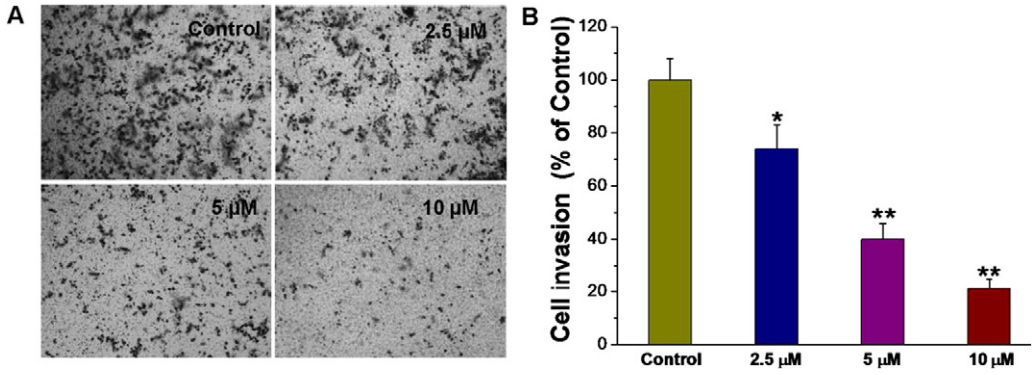

Fig. 2. (A) Effects of **Ir1** on MDA-MB-231 cells invasion in Boyden chamber invasion assay. (B) Invasion cells after treated with different concentration (2.5, 5, and 10 μM) of **Ir1** for 24 h. Invasion cells were quantified and expressed on 100% (% of control). * = $p < 0.05$; ** = $p < 0.01$, compared with the control group.

that of the control cells, and **Ir1** treatment increased the wound size in a dose-dependent manner (Fig. 1C). Therefore, **Ir1** significantly blocked MDA-MB-231 cell migration.

In addition to migration, invasion is another critical component of the metastatic process. To determine whether **Ir1** could reduce tumor invasion, the Boyden chamber invasion assay was employed. A layer of Matrigel (BD Biosciences) was added over the membrane located on the top of the chamber to evaluate the invasion of MDA-MB-231 cells. Cells that invaded the Matrigel and migrated to the bottom of the chamber membrane were labeled using crystal violet dye [12]. A large number of cells were dyed with crystal violet, indicating that intensive cell invasion occurred. After treatment with **Ir1** for 24 h, the number of MDA-MB-231 cells that penetrated the Matrigel decreased significantly compared to the control group (Fig. 2A). The results revealed that **Ir1** caused a concentration-dependent decrease in MDA-MB-231 cell invasion (Fig. 2B).

The antiproliferative effect of **Ir1** on MDA-MB-231 cells and L02, a normal liver cell line, was evaluated using the MTT assay. The results showed that **Ir1** exhibited potent toxicity against tumor cell line MDA-MB-231 but was significantly less toxic to the normal liver cell line L02, which indicated that **Ir1** would likely present a good therapeutic profile (Fig. 3A). Most of the readily available cytotoxic chemical-based drugs mediate their effects by inducting apoptosis in cancer cells. To further analyze the mechanism of **Ir1**-induced cell death, the involvement of apoptosis in cell death was determined using annexin V-FITC/PI double staining assays. As shown in Fig. 3B, exposure of MDA-MB-231 cells to **Ir1** led to a marked and dose-dependent increase in the proportion of apoptotic cells. Hence, the cytotoxic effects of **Ir1** on

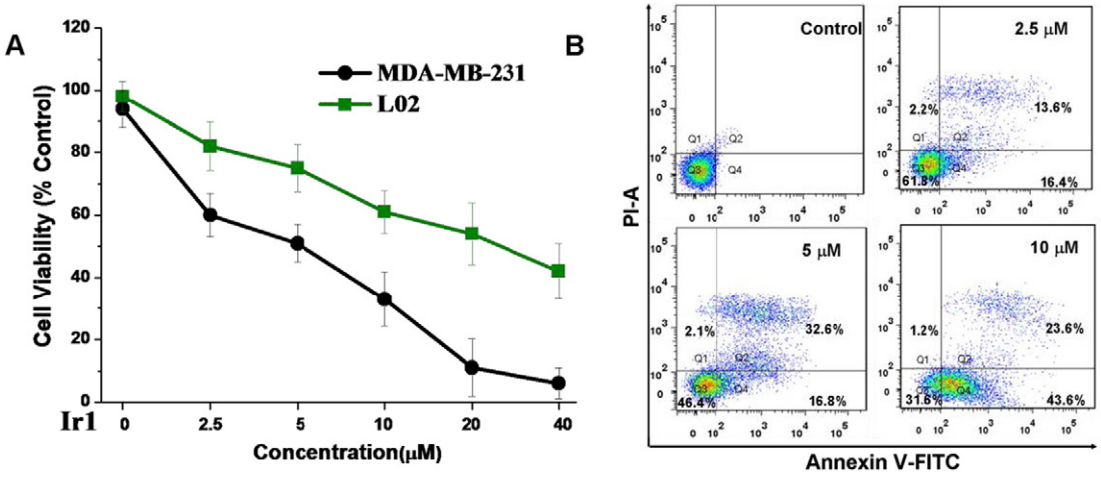

Fig. 3. (A) Cytotoxicity of **Ir1** on MDA-MB-231 and L02 cell line. (B) **Ir1** induced apoptosis was examined by the annexin V-FITC/PI assay. The percentages of cells in each quadrant are shown (Q2: late apoptotic or necrotic cells, Q4: early apoptotic cells).