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Insights into the mevalonate pathway in the anticancer effect of a platinum complex on human gastric cancer cells

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ABSTRACT

A platinum(II) complex $[Pt(en)]_2ZL$ [en = ethylenediamine; ZL = 1-hydroxy-3(1H-imidazol-1-yl)ethane-1,1-diylbisphosphonic acid, commonly called as zoledronic acid] has been designed and synthesized recently in order to look for new anticancer drugs with high efficacy and low side effects. It exhibited cytotoxic effects on the human cancer cells SGC7901, HepG2, MCF-7, MDA-MB-231, HCT116, and U2OS, and the cytotoxicity against SGC7901 is particularly remarkable. It also showed higher cytotoxicity and better selectivity than the corresponding ligand ZL in inhibiting cancer cells SGC7901 and HepG2 rather than normal cells GES-1 and LO2. To investigate the role of mevalonate pathway involved in the mechanism of anticancer action of $[Pt(en)]_2ZL$, the effects of farnesol (FOH) and geranylgeraniol (GGOH), precursors of important mevalonate pathway intermediates farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), on the cytotoxic effects of $[Pt(en)]_2ZL$ against the human gastric cancer cells SGC7901 were investigated systematically, since inhibition of the key enzyme farnesyl pyrophosphate synthase (FPPS) in the mevalonate pathway was acknowledged as the mechanism of most anticancer actions of the ligand ZL. The experiments revealed that FOH and GGOH both rescued the SGC7901 cells, especially FOH. The cell cycle arrest and apoptosis of SGC7901 cells induced by $[Pt(en)]_2ZL$ was decreased by the addition of FOH, and the prenylation of small guanine-nucleotide-binding regulatory proteins (small G proteins) down-regulated by $[Pt(en)]_2ZL$ was recovered by the addition of FOH, demonstrating that $[Pt(en)]_2ZL$ exerted anticancer effects on SGC7901 via inhibiting the mevalonate pathway. This will provide deep insights into the mechanism of action of $[Pt(en)]_2ZL$.

1. Introduction

Increasing incidence of cancers is emerging globally and has become a seriously public health problem. Gastric cancer is reported to be one of the most common cancers worldwide, which accounts for about 8% of total cancer cases and 10% of total deaths from cancer (Quéro et al., 2015). Up to now, surgery is still the main treatment method for gastric cancer, especially for those patients in early stage (Zhang et al., 2015). To improve the survival rate of patients with gastric cancer, potent chemotherapeutic strategies have also received considerable attention (Dittmar and Settmacher, 2015). Over the past decades, a variety of metal complexes have been used in the anticancer therapy. In particular, cisplatin has been acknowledged as one of the most widely-used drugs in treating several cancers (Kelland, 2007). However, various side effects due to lack of specificity and intrinsic or acquired drug resistance often limited its clinical applications (Johnstone et al., 2013; Kim and Park, 2015). Therefore, in order to improve the chemotherapy efficacy and reduce the side effects, enormous effort has been dedicated to synthesizing various novel metal complexes. A number of delivery vehicles have also been exploited to design and prepare new anticancer agents with better specificity (Dancey and Chen, 2006). For example, bisphosphonates (BPs) have been used widely as the drug targeting and drug delivery carriers for therapeutic and diagnostic applications, due to the fact that they possess strong affinity for bone mineral and other calcified tissues (de Rosales et al., 2009; Palma et al., 2011). Recently, we have also employed the nitrogen-containing bisphosphonates (N-BPs) as the ligands to synthesize a series of novel platinum complexes, which exhibited remarkable antiproliferative effects on several cancer cells (Qiu et al., 2015). In particular, the complex $[Pt(en)]_2ZL$ (Fig. 1)

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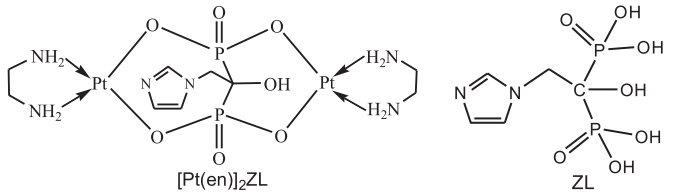

Fig. 1. Chemical structures of the complex $[Pt(en)]_2ZL$ and the ligand ZL.

exerted remarkable anticancer effect on the human gastric cancer cells SGC7901 (Yang et al., 2016).

Up to now, a plenty of studies have demonstrated that N-BPs, especially ZL (Fig. 1), not only can inhibit the bone resorption but also can exhibit excellent antitumor effects (Russell, 2011). And increasing evidence has also shown that the pharmacological effects of N-BPs are attributed to their specificity to inhibit the key enzyme farnesyl pyrophosphate synthase (FPPS) in the mevalonate pathway (Fujita et al., 2012; Goffinet et al., 2006; Reinholz et al., 2002; Tsoumpra et al., 2015). Inhibition of FPPS will deplete the formation of essential mevalonate pathway intermediate isoprenoids, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (Goffinet et al., 2006; Thurnher et al., 2012), which are necessary for the post-translational prenylation of several small G proteins, such as Rho and Ras (Jones et al., 2015; Thurnher et al., 2012). Subsequently, the cellular signal transduction will be affected, thereby leading to the cell cycle arrest, apoptosis, and so on. As a result, the mevalonate pathway is fundamental for the cell growth and survival, and therefore it has become one important target for developing novel anticancer drugs.

Although the complex $[Pt(en)]_2ZL$ prepared by us recently based on the N-BPs ligand ZL showed remarkable anticancer effects on several cancer cells by inducing cell cycle arrest and apoptosis (Qiu et al., 2015; Yang et al., 2016), its mechanism of action is still less clear and unexplored largely. Therefore, it is essential to further investigate the mechanism of anticancer actions of $[Pt(en)]_2ZL$. In the present work, in order to investigate the role of mevalonate pathway in the anticancer actions of $[Pt(en)]_2ZL$, the effects of farnesol (FOH) and geranylgeraniol (GGOH), cell permeable precursors of important mevalonate pathway intermediates FPP and GGPP, on the cell proliferation, cell cycle arrest, and cell apoptosis of SGC7901 as well as unprenylation of small G proteins induced by $[Pt(en)]_2ZL$ have been studied.

2. Materials and methods

2.1. Chemicals and reagents

$[Pt(en)]_2ZL$ was provided by our lab (Qiu et al., 2015) and dissolved in phosphate buffer saline (PBS) to give the stock solution (4×10^3 μM), and stored at 4 °C. Farnesol (FOH) was purchased from Energy Chemical Co. (Shanghai, China) and geranylgeraniol (GGOH) was purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). They were both dissolved in 70% ethanol to give the stock solution (4×10^3 μM and 3×10^3 μM, respectively) and stored at 4 °C. The reagents 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and bicinchoninic acid (BCA)-protein-assay kit were purchased from Solarbio Science & Technology (Shanghai, China). The fluorescein isothiocyanate (FITC)-Annexin V/propidium iodide (PI) apoptosis assay kit was purchased from Nanjing KeyGEN Biotech (Jiangsu, China). Hoechst 33342, RNase A, Triton X-100, electrochemiluminescence (ECL) and PI dye were purchased from Beyotime (Shanghai, China). Monoclonal antibodies specific to poly(ADP-ribose) polymerase (PARP), cleaved-caspase 3, 8, 9 and Cyclin D1 were purchased from Cell Signal Technologies (Beverly, MA, USA). Ras and RhoA antibodies were purchased from Abcam Trading Co., Ltd (Shanghai, China). The secondary antibodies to anti-mouse and anti-rabbit immunoglobulin G (IgG)-conjugated horseradish peroxidase (HRP) were purchased from Bioworld Technology (Minneapolis, MN, USA).

2.2. Cell culture

Human gastric cancer cell lines SGC7901, human hepatocarcinoma cell lines HepG2, human estrogen receptor positive breast cancer cell lines MCF-7, human estrogen receptor negative breast cancer cell lines MDA-MB-231, human colon carcinoma cell lines HCT116, and human osteosarcoma cell lines U2OS as well as the human normal gastric mucosal epithelial cell lines GES-1 and human normal liver cell lines LO2 were obtained from the Cell Bank of Chinese Academy of Science (Shanghai, China) and maintained in HG-Dulbecco modified Eagle's medium (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 10% (v/v) fetal bovine serum (FBS, Biological Industries, Kibbutz Beit Haemek, Israel), 100 U/ml penicillin, and 100 μg/ml streptomycin. They were incubated in a 37 °C incubator (Thermo Electron Corporation, USA) with a humidified atmosphere containing 5% CO_2.

2.3. Cytotoxicity assay

The cytotoxicity of $[Pt(en)]_2ZL$ against the cancer cells and normal cells was investigated by the conventional MTT assay. Briefly, cells (5×10^4 cells/ml) were seeded in 96-well plates with 100 μl culture medium per well. After an overnight incubation to allow cells attachment, the medium was removed and replaced by the increasing concentration of the drug $[Pt(en)]_2ZL$. Then, the plates were incubated at 37 °C in 5% CO_2 for 48 h. Before next processing, the cells were observed under the microscope and photographed by a Panasonic Lumix DMC-FH2. Then, MTT solution (5 mg/ml, 20 μl) was added to each well. After plates were cultured at 37 °C for another 4 h, the supernatants were removed and DMSO (150 μl) was added to each well for dissolving the formazan crystal. The absorbance was measured at the wavelength of 490 nm using a microplate reader (BioTek Instruments, Inc. Vermont, USA). The IC_{50} values (the concentration of the drug used for inhibiting the cell growth by 50%) were determined from the dose-response curve according to the inhibition rate at each concentration. The results shown were representative of at least three independent experiments (mean ± SD). For better comparison, the cytotoxicity of the corresponding ligand ZL against the cancer cells and normal cells were also investigated in parallel. In addition, the effects of exogenous FOH and GOOH on the antiproliferative effect of $[Pt(en)]_2ZL$ were examined by treating together with exogenous FOH (10 μM) or GOOH (10 μM).

2.4. Cell cycle analysis

According to the MTT assay, $[Pt(en)]_2ZL$ showed the strongest inhibition effect on the proliferation of SGC7901 cells. Therefore, the cancer cell line SGC7901 was selected for further study. SGC7901 cells were seeded at a density of 2×10^5 cells/ml per well in 6-well plates and incubated at 37 °C overnight to allow cells attachment. After incubation with 30 μM of $[Pt(en)]_2ZL$ for 48 h, cells were harvested, washed with ice-cold PBS and fixed with 70% ethanol at 4 °C overnight. The fixed cells were washed and resuspended in 200 μl PBS containing PI (50 μg/ml) and RNase A (50 μg/ml). Then, the samples were incubated at 37 °C for 30 min in the dark and analyzed for DNA content by flow cytometry (FCM) (FACSCalibur, Becton Dickinson, USA), and the populations of G0/G1, S, and G2/M phases were quantified using FlowJo software as previously described. Effect of FOH on the cell cycle distribution induced by $[Pt(en)]_2ZL$ was examined by treating together with exogenous FOH (10 μM).