Biomaterials 71 (2015) 168–177

Contents lists available at ScienceDirect

Biomaterials

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

Cellular localization of iron(II) polypyridyl complexes determines their anticancer action mechanisms

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
13 August 2015
Accepted 16 August 2015
Available online 18 August 2015

Keywords:
Iron(II) polypyridyl complexes
Cellular localization
Cellular uptake
Action mechanisms

ABSTRACT

Elucidation of relationship among cellular uptake, localization and biological activities of metal complexes could make great breakthrough in the understanding of their action mechanisms and provide useful information for rational design of metal-based anticancer drugs. Iron(II) complexes have emerged as potential anticancer drug candidates with application potential in cancer imaging and therapy. Herein, a series of iron(II) polypyridyl complexes with different lipophilicity were rationally designed, synthesized and identified as potent anticancer agents. The relationship between the cellular localization and molecular action mechanisms of the complexes was also elucidated. The results showed that, the increase in planarity of the Fe(II) polypyridyl complexes enhanced their lipophilicity and cellular uptake, leading to improved anticancer efficacy. The hydrophilic Fe(II) complex entered cancer cells through transferring receptor (TfR)-mediated endocytosis, and translocated to cell nucleus, where they induced S phase cell cycle arrest through triggering DNA damage-mediated p53 pathway. Interestingly, the hydrophobic Fe(II) complexes displayed higher anticancer efficacy than the hydrophilic ones, but shared the same uptake pathway (TfR-mediated endocytosis) in cancer cells. They accumulated and localized in cell cytoplasm, and induced G0/G1 cells cycle arrest through regulation of AKT pathway and activation of downstream effector proteins. These results support that the cellular localization of Fe(II) complexes regulated by their lipophilicity could affect the anticancer efficacy and action mechanisms. Taken together, this study may enhance our understanding on the rational design of the next-generation anticancer metal complexes.

1. Introduction

Iron (Fe) plays a major role in many crucial biological systems, including DNA synthesis, cell growth and proliferation [1]. Studies have shown that three-dimensional Fe complexes could be tailored by modification of the ligands to improve their application as disease therapeutics and luminescent probes for biomolecules [2,3]. Most importantly, the Fe(II) complex of the glycopeptide bleomycin (Fe(BLM)) that can cause double-strand breaks in DNA, is clinically used in cancer treatment [4,5]. This success has motivated investigation of new Fe(II/III) complexes for the treatment of cancer. Till now, a lot of Fe complexes have been synthesized and identified as antiproliferative agents against cancer growth, such as the Fe(II/III) chelating complexes, ferrocenyl-containing complexes and Fe(II/III) polypyridyl complexes [6–8]. For instance, Amatore and his coworkers prepared a series of hydroxyferrocifens and found that the complexes presented a remarkable anti-proliferative behavior on breast cancer cells [9]. Meanwhile, Easmon et al. synthesized a series of iron(II) complexes of thiosemicarbazones that demonstrated prominent antitumor activity [10]. Moreover, studies also found iron(III) complexes showed photocytotoxic and could be developed as cell imaging agents in near-IR light [11]. Chakravarty et al. found dipyridophenazine iron(III) complexes could be potent photocytotoxic agents in visible light [12]. Based on these findings, the search for next-generation Fe(II/III) complexes as cancer theranostic agents has kindled great interest of scientists from chemistry and medicine.

Metal complexes display superior physiochemical properties, such as large Stoke shifts for emitting luminescence and thus demonstrate application potential as molecular imaging [13,14]. Notwithstanding, many functional imaging techniques have played an important role in cancer diagnostics, such as computed tomography, positron emission tomography, radiography and magnetic

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http://dx.doi.org/10.1016/j.biomaterials.2015.08.031
0142-9612/

resonance imaging. Luminescence molecular imaging not only provides high sensitivity and specificity in tumor detection, but also guides the precise intraoperative positioning [15]. Thus, it is not surprising that more and more research focus on the metal complexes which can be used for molecular imaging and theranostic [2,16–22]. Recently, Ir(II)/(III) complexes were developed as the luminescence cellular imaging probes for specific mitochondrial imaging and tracking [23–27]. Barton and Gilles also found that Ru(II) polypyridyl complexes not only exerted biological effects but also could be the luminescence cellular imaging probes, specially for RNA mismatches [18,28–30]. Meanwhile, Che et al. found that Au(III) complexes containing N-heterocyclic carbene ligands could be the thiol "switch-on" fluorescent probes and anti-cancer agents [31], while Parker et al. observed the selective staining of chromosomal DNA in dividing cells using a luminescent Tb(III) complex [32]. Importantly, Vicente et al. found that the intracellular localization of the Zn(II)-phthalocyanines determined their photodynamic activity [33]. However, relationship between their anticancer efficacy and localization need further elucidation. Moreover, little information about the application of anticancer Fe complexes in molecular imaging and cancer diagnosis, and the underlying molecular mechanisms is available. Studies on this topic may provide useful information for further design of metal-based cancer theranostic agents.

The lipophilicity of metal complexes is well-known to have a strong influence on its cellular uptake and intracellular translocalization [30]. Studies have shown that, information on the cellular localization of metal complexes not only provides further insight into action mechanisms, but also offers the possibility for development of next-generation theranostic agents [34]. For these reasons, it is not surprised that much attentions have been paid to the cellular localization of metal complexes. For example, Gasser et al. reported that the more lipophilic Ru(II) complexes incorporating 2-pyridyl-2-pyrimidine-4-carboxylic acid specifically targeted mitochondria and induced apoptosis in HeLa cells [30]. Studies also reported that Ru(II) dipyridophenazine complexes ~~can~~ could mainly accumulate in the cytoplasm of live cells but were mostly excluded from the nucleus [35]. Our previous study also presented that the lipophilicity Ru(II) polypyridyl complexes located in mitochondria and induced apoptosis in cancer cells [36,37]. Besides Ru(II) complexes, other metal complexes, such as Pt(II), Au(III), Cu(I) and Ir(III) have been investigated for their relationship between cellular localization and anticancer action mechanisms [31,38–40]. Nevertheless, there are barely reports on the relationship of Fe(II) polypyridyl complexes which illuminate the important role of cellular localization in cancer treatment. Therefore, to design some new Fe(II) polypyridyl complexes and study their cellular localization and antitumor action mechanisms would be significant. Inspired by the these findings, in this study, we rationally designed, synthesized a series of iron(II) polypyridyl complexes with different lipophilicity and identified them as potent anticancer agents. The relationship between the cellular localization and molecular action mechanisms of the complexes was also elucidated. The results showed that, hydrophilic Fe(II) complex entered cancer cells through transferring receptor (TfR)-mediated endocytosis, and translocated to cell nucleus, where they induced S phase cell cycle arrest through triggering DNA damage-mediated p53 pathway, while the hydrophobic Fe(II) complexes localized in cell cytoplasm, and induced G0/G1 cells cycle arrest through regulation of AKT pathway and activation of downstream effector proteins. These results supported that the cellular localization of Fe(II) complexes affected by their lipophilicity could affect the anticancer efficacy and action mechanisms. Taken together, this study may enhance our understanding on the rational design of the next-generation anticancer metal complexes.

2. Material and methods

2.1. Materials and general instruments

$Fe(NH_4)_2(SO_4)_2\cdot 6H_2O$, $NaClO_4$, cisplatin and ligands 2, 2'-bipyridine (bpy), 1,10-phenanthroline (phen), imidazole[4,5-f][1,10] phenanthroline (ip), 2-phenylimidazo[4,5-f][1,10]phenanthroline (pip), 2-(4-methylphenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (me-pip) were purchased commercially and used without further purification. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), BCA assay kit were purchased from Sigma–Aldrich. All of the antibodies used in this study were purchased from Cell Signaling Technology (Beverly, MA, USA). In all experiments, organic solvents were analytical grade unless otherwise stated.

2.2. Synthesis and characterization of the Fe(II) complexes

2.2.1. Synthesis of $Fe(bpy)_3(ClO_4)_2$ (**1**) and $Fe(phen)_3(ClO_4)_2$ (**2**)

The Fe(II) complexes **1**–**2** were synthesized according to published procedures [41].

2.2.2. Synthesis of $Fe(ip)_3SO_4$ (**3**), $Fe(pip)_3SO_4$ (**4**) and $Fe(Me\text{-}pip)_3SO_4$ (**5**)

Complexes **3**–**5** were prepared using a modified synthetic procedure in which 5 mL of an aqueous solution of $Fe(NH_4)_2(SO_4)_2\cdot 6H_2O$ (0.2 g, 0.5 mmol) was added drop-wise to 20 mL of a ethanol 95% solution of the diimine ligands (ip, 0.33 g; pip, 0.47 g; me-pip, 0.47 g; 1.5 mmol). The solution was stirred for 40 min, filtered and the deep red solid of the sulfate salt as the product (complexes **3**–**5**) washed with ethanol 95% and finally dried in vacuum drying oven. The products were then purified by alumina column chromatography with toluene and methanol as eluants.

The Fe(II) complexes **1**–**5** showed good solubility in methanol, ethanol, acetonitrile, chloroform, dichloromethane, DMSO, DMF and slightly worse solubility in water.

$Fe(ip)_3SO_4$ (3) Yield 80%. ESI-MS: *m/z* 358.4 $[M\text{-}SO_4^{2-})]^{2+}$. Elemental analysis calc (%) for $C_{39}H_{24}N_{12}O_4SFe$: Elemental analysis: C, 57.64; H, 2.98; N, 20.68; found (%): C, 57.54; H, 2.90; N, 20.60. UV–Vis (λ (nm), $\varepsilon/10^4$ (M^{-1} cm^{-1}): 251 (11.37), 281 (7.26), 520 (1.07). IR (KBr): ***ν*** 3074 (N–H), ***ν*** 1585, 1453 (C = C_{arom}) cm^{-1}. ^1H NMR (DMSO-d_6, δ ppm): 8.81 (d, 6H), 8.06 (s, 6H), 7.75 (t, 6H), 7.36 (d, 6H). ^{13}C NMR (DMSO-d_6, δ ppm): 152.71 (s, 3C), 148.85 (s, 3C), 146.67 (s, 9C), 137.85 (s, 6C), 129.53(s, 6C), 127.53(s, 6C), 125.01(s, 3C), 124.33(s, 3C).

$Fe(pip)_3SO_4$ (4) Yield 80%. ESI-MS: *m/z* 472 $[M\text{-}SO_4^{2-})]^{2+}$. Elemental analysis calc (%) for $C_{57}H_{36}N_{12}O_4SFe$: C, 65.77; H, 3.49; N, 16.15; found (%): C, 65.70; H, 3.41; N, 16.08. UV–Vis (λ (nm), $\varepsilon/10^4$ (M^{-1} cm^{-1}): 286 (10.37), 534 (1.96). IR (KBr): ***ν*** 3059 (N–H), ***ν*** 1590, 1460 (C = C_{arom}) cm^{-1}. ^1H NMR (DMSO-d_6, δ ppm): 8.96 (d, 6H), 8.36 (d, 6H), 7.62 (d, 6H), 7.48 (d, 6H), 7.32 (t, 6H).

$Fe(Me\text{-}pip)_3SO_4$ (5) Yield 84%. ESI-MS: *m/z* 493.4 $[M\text{-}SO_4^{2-})]^{2+}$. Elemental analysis calc (%) for $C_{40}H_{42}N_{12}O_4SFe$: C, 66.77; H, 3.91; N, 15.52; found (%): C, 66.70; H, 3.81; N, 15.48. UV–Vis (λ (nm), $\varepsilon/10^4$ (M^{-1} cm^{-1}): 288 (6.25), 534 (0.86). IR (KBr): ***ν*** 3074 (N–H), ***ν*** 1610, 1460 (C = C_{arom}) cm^{-1}. ^1H NMR (DMSO-d_6, δ ppm): 8.98 (d, 6H), 8.26 (s, 6H), 7.96 (s, 3H), 7.66 (m, 6H), 7.33 (d, 6H), 2.38 (s, 9H).

2.3. Measurement of lipophilicity (lipo-hydro partition coefficient)

The lipophilicity of complexes **1**–**5** was determined by using the "shake-flask" method previously reported [31]. The concentrations of the Fe(II) complexes in each phase were determined by UV–Vis analysis. Log*P* was calculated as the logarithmic ratio of the