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REVIEW

Iron chelates in the anticancer therapy

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Abstract
Iron plays a significant role in the metabolism of cancer cells. In comparison with normal cells, neoplastic ones exhibit enhanced vulnerability to iron. Ferric ions target tumor via the ferroptotic death pathway—a process involving the iron-mediated lipid oxidation. Ferric ion occurs in complex forms in the physiological conditions. Apart from iron, ligands are the other factors to affect the biological activity of the iron complexes. In recent decades the role of iron chelates in targeting the growth of the tumor was extensively examined. The ligand may possess a standalone activity to restrict cancer's growth. However, a wrong choice of the ligand might lead to the enhanced cancer cell's growth in in vitro studies. The paper aims to review the role of iron complex compounds in the anticancer therapy both in the experimental and clinical applications. The anticancer properties of the iron complex rely both on the stability constant of the complex and the ligand composition. When the stability constant is high, the properties of the drug are unique. However, when the stability constant remains low, both components—ferric ions and ligands, act separately on the cells. In the paper we show how the difference in complex stability implies the action of ligand and ferric ions in the cancer cell. Iron complexation strategy is an interesting attempt to transport the anticancer $Fe^{2+/3+}$ ions throughout the cell membrane and release it when the pH of the microenvironment changes. Last part of the paper summarizes the results of clinical trials and in vitro studies of novel iron chelates such as: PRLX 93,936, Ferumoxytol, Talactoferrin, DPC, Triapine, VLX600, Tachypyridine, Ciclopiroxamine, Thiosemicarbazone, Deferoxamine and Deferasirox.

Keywords Iron chelates · Iron complex · Cancer · Chemotherapy · Diet supplements

Introduction

Anticancer therapy aims to induce apoptosis in cancer cells, simultaneously leaving healthy ones unaffected. However, common defects in the executioner mechanism of cell death lead to the resistance to the therapy and make treatment process very elaborate and sometimes unpredictable. Nowadays, researchers aim to enhance the cytotoxic activity of the common chemotherapeutics by combining them with other anticancer compounds (Qi et al. 2017).

Unimpeded growth of cancer is promoted by various factors—including iron accessibility. Thus, neoplastic cells exhibit the iron-seeking phenotype by substantially increasing the demand for the microelement in comparison with healthy cells (Richardson et al. 2009). The phenomenon is induced by the dysregulation of the proteins related to iron metabolism. Iron cellular hemostasis is partially mediated by tumor suppressor genes and oncogenes (Manz et al. 2016). The hallmark makes cancer cells vulnerable to ferroptosis—a non-apoptotic nor necrotic type of cellular death (Tang and Kroemer 2020). The process includes lipid peroxidation as the result of Fenton's reaction (see Fig. 1). Therefore, high concentration of iron (II) ions in the cytoplasm of the cells enhances the process (Xie et al. 2016). Nowadays, ferroptosis is examined as the potential tool in the anticancer therapy.

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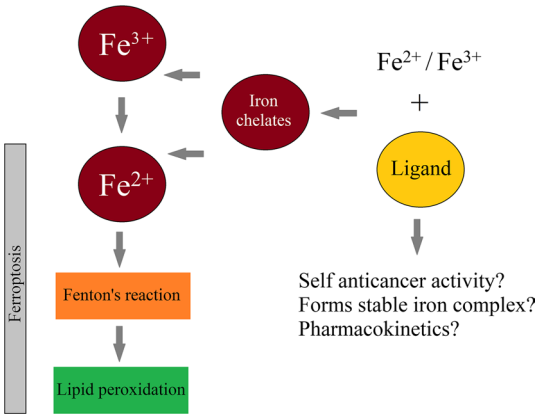

Fig. 1 The role of ligand properties and stability of iron complex in the induction of ferroptosis

Even though the process has already been extensively characterized, it remains not fully clear. One of the confusing points is the role of frataxin in the process (Du et al. 2020). Inside cells, frataxin acts as an iron buffer, protecting them from iron overload and also supporting the cells' energy metabolism by enhancing the assembly of proteins which contain Fe-S clusters. The decrease in frataxin's expression enhances ferroptosis (Du et al. 2020).

Quantity and the site of iron ions' administration turn out to be crucial when it comes to its influence on carcinogenesis. On the one hand, the excess of iron leads to increased cancer risk, cancer initiation and tumor growth (Knekt et al. 1994). Conversely, the depletion of the microelement could be effectively used in treatment (Torti et al. 2018).

Ferric chelates are used as oral iron supplements as well as phosphate binders. The potential of iron (III) citrate and iron (III) EDTA complex in chemotherapy is currently being examined and yet show several ambiguous results. On the one hand, the iron (III)-EDTA decreases the expression of frataxin in colon adenocarcinoma, which leads to the induction of ferroptosis (Schulz et al. 2006). On the other hand, iron (III) citrate induces the progression of cancer, therefore cannot be considered as a potential therapeutic option (Scheers et al. 2018). Relatively low stability of iron (III) citrate leads to the delivery of citrate anions to cancer cells, which increases the Krebs cycle rate and therefore promotes progression (Scheers et al. 2018). Understanding the role of iron in the biology of cancer cells and the pathways of its significant influence might be crucial for the future examinations on therapy prospects. However, it is not yet well understood why some forms of iron mobilize and accelerate the cancer cells (Buss et al. 2005a; b).

The paper aims to overview the potential of iron complex compounds in the anticancer therapy. In the beginning we explain the mechanisms in which ferric ions induce apoptosis in cancer cells. Then we present the latest laboratory studies concerning the anticancer properties of iron chelates. Next, we move to the effects of the currently used diet supplements on the carcinogenesis process and explain the role of iron chelates stability on the safety of the medication. In the end we move to the clinical trials involving the use of iron compounds.

For the review paper, we have searched Google Scholar for most recent papers about the application of iron compounds in the anticancer therapy. We used only peer-reviewed papers from journals in the fields of medicine, biology or chemistry. Due to the focus of the paper on the potential of iron chelates in the anticancer therapy, we mostly omitted papers regarding the free iron's role. Also, our search of papers included only the well-studied compounds. As for the calculations of concentrations of the ligand and various iron forms, we omitted the ionic strength and worked simply on molar concentrations.

Iron-mediated cytotoxicity against cancer

Markedly elevated concentration of iron increases overall death risk, including the elevated risk of cancer. Iron is responsible for catalyzing ROS production. Furthermore, it provides survival and progression of cells, which may have progressed to malignancy. Iron redox cycle is associated with reactive oxygen species (ROS) production. Hydroxyl radical is one of Fenton's reaction product (see below).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\cdot} + H^{+}$$

$$2H_2O_2 \rightarrow HO^{\cdot} + HOO^{\cdot} + H_2O$$

"Free" iron—non-transferrin plasma iron, consists of both Fe^{3+} and Fe^{2+}. Iron on the III oxidative state is more stable in physiological conditions than iron on the second oxidative state (II). In neutral pH most Fe^{3+} precipitates as hydroxides. Some of Fe^{3+} chelates with low molecular weight chemical structures (ATP, ATP and GTP) stay as "free" iron in neutral pH. The fewer ligands are involved in chelation, the higher is the catalyzing activity of ferric ions to produce $OH^{\cdot}$ radicals. Reducing agents (e.g., ascorbate, superoxide dismutase, glutathione) protect cells from the influence of ROS and Fenton's reaction (Toyokuni 1996).

Peroxidative damage, spread by ROS, includes the injury of phospholipids from organelle's membranes. Iron induces membrane disruption and distortion that alters the activity of the cell. These effects lead to the destruction of organelles such as mitochondria, microsomes or lysosomes. Iron is involved in the loss of cellular viability, by increasing phospholipase A (PLA) and lysophosphatidic activity (Bacon and

Britton 1990). Interestingly, iron-citrate complex efficiently induces DNA strand breaks (Toyokuni and Sagripanti 1993).

Tumoricidal action of macrophages is essential in inhibiting malignancy progression. Therefore sadly, iron dextran, iron salts, carbonyl iron and iron ferritin were reported to suppress anticancer activity of macrophages (Green et al. 1988). Interestingly, tumor cells express a large number of transferrin receptors.

Iron complexes in the anticancer therapy under in vitro and in vivo evaluation

Initially, iron chelators were developed to treat iron overload, now these agents are being repurposed to treat cancers. The agents have not yet been classified as anticancer drugs. There could be distinguished two main strategies of iron chelates' action. The first one includes depletion of iron from cancer cells by the inhibition of cellular iron uptake or the promotion of iron metabolism. The second option focuses on the application of iron chelates to facilitate the redox cycling of iron, to generate cytotoxic reactive oxygen species (ROS) within tumor (Torti and Torti 2013). The other studies concern the influence of iron chelators on the inhibition of ribonucleotide reductase, which limits the iron-dependent enzymes for DNA synthesis or on excitation of cell cycle arrest (Lane et al. 2014). Studies concerning ferric chelates include Deferasirox (DFX), Deferoxamine (DFO), Thiosemicarbazone, Ciclopiroxamine (CPX), Tachypyridine, VLX600, citrate and EDTA.

Deferoxamine (DFO) and Deferasirox (DFX) display significant antiproliferative activity in human gastric cell lines. It is explained by the induction of G1 phase arrest and apoptosis. Apoptosis is induced by increased reactive oxygen species (ROS) production and c-Jun N-terminal kinase (JKN) activation (Kim et al. 2016).

In vitro and in vivo studies proved low toxicity of iron chelators and high efficiency in inhibiting tumor cell proliferation (Kicic et al. 2001). DFO, which showed promising antiproliferative properties on cancer cell lines, also demonstrated in vivo a dose-dependent antileukemic effect through the action on proliferation and differentiation of blasts (Estrov et al. 1987; Dezza et al. 1989). Furthermore, DFO promoted apoptosis of cancer cells in mammary adenocarcinoma-bearing rats. A low iron diet led to similar results indicating iron depletion as the mechanism of antitumor action of DFO (Wang et al. 1999; Jiang et al. 2002).

In the study by Saeki, DFX was proven to inhibit the proliferation of three hepatoma cell lines (HepG2, Hep3B and Huh7) in a dose-dependent manner (Saeki et al. 2016). Moreover, it induced apoptosis, by increasing caspase-3 activity (Saeki et al. 2016). DFX up-regulates mRNA expression of hepcidin, transferrin receptor 1 and HIF-1α levels. This leads to the inhibition of tumor growth via hypoxia-associated factors. Although DFX inhibited the proliferation of hepatoma cell lines and induced the activation of caspase-3 in vitro, the results were not supported by clinical studies.

In vitro antiproliferative activity of Thiosemicarbazone demonstrated an anticancer activity by increasing the expression of the growth and metastasis suppressor N-myc downstream-regulated gene 1 and its phosphorylation at Ser330 and Thr346. Furthermore, the agent augmented the expression of the cyclin-dependent kinase inhibitor p21, whilst decreasing cyclin D1 in pancreatic cells (Kovacevic et al. 2011). Several studies demonstrated potent inhibition of ribonucleotide reductase (RNR), which led to the reduced DNA replication and repair as an answer to Thiosemicarbazone activity (Finch et al. 2000).

Ciclopiroxolamine (CPX), a fungicidal with anticancer activity, is mediated through iron chelation and subsequent inhibition of iron-dependent enzymes. CPX downregulated DJ-1 (endogenous antioxidant oncogene), leading to ROS accumulation, impairing mitochondrial function and inducing apoptosis. Although in vitro studies were promising, the clinical use was abandoned because of the poor solubility of the drug, as well as its rapid metabolism into inactive glucuronide and quick clearance from the body (Weir et al. 2019).

A novel molecule VLX600 similar to CPX and DFO has been also classified as an iron chelator. VLX600 exhibits anticancer activity—in vitro by inhibiting RNA reductase of proliferating cells, decrease mitochondrial oxidative phosphorylation and eventually leads to decreased production of mitochondrial energy. Furthermore, VLX600 has proven to be more firm than other iron chelators in antiproliferative activity on colon cancer cells line.

Tachypyridine induces cell cycle arrest in the G2 phase in the HeLa cell line (cervical cancer) and CRC cell line (colorectal cancer). Interestingly, a non-iron analogue did not exhibit the same activity, suggesting the iron-related therapeutic activity (Brown et al. 2020).

Effects of iron complexes used as diet supplements on cancer

Currently, two iron-containing diet supplements are used by the patients. Ferric citrate and ferric-EDTA aim to increase total iron in patients' organism; however, treatment with the drugs was reported to induce wide biological effects. Various forms of iron chelators impact tumorigenesis differently. Ferric citrate and ferric EDTA were discovered to promote colon cancer in mice. Further in vitro research proved that cells incubated with high concentration (0.5–2 mM) of ferric citrate induced amphiregulin (oncogenic growth factor) and its receptor EGFR (epithelial growth factor receptor) production and the activation of mitogen-activated protein kinase (MAPK), which lead to an increased risk of cancer progression. The dose of 0.5 mM ferric EDTA triggered the

same up-regulation of amphiregulin, while other iron chelators such as ferrous sulfate did not evoke any. Furthermore, the smaller dose (0.05 mM) of both ferric citrate and ferric EDTA did not target amphiregulin (Scheers et al. 2018). Amphiregulin, as a growth factor, induces proliferation and inhibit apoptosis. Abnormal activity stimulated by iron chelators of amphiregulin leads to epithelial cancer development (Kariagina et al. 2010).

The study performed by Poljak-Blazi compared the influence of ferric-sorbitol-citric complex on the ROS production and proliferation in cervical carcinoma cell lines (HeLa, SiHa) and human papillomavirus (HPV) cell line (Poljak-Blazi et al. 2011). The cells were treated with Fe (III) ions at a concentration between 0.001 and 1 mM for various periods of time. As it was expected—some of the measurements exhibited positive outcome, it was also proven that Fe (III) ion treatment enhances survival of HPV 16 positive cells and may improve HPV oncogenesis.

Loreto et al. proved the antitumor activity of standalone EDTA on cancer cells of various lines. Efficacy varied between cell lines. Moreover, the toxicity of EDTA was higher in melanoma cells compared to normal melanocytes. The observation confirms that cancer cells are characterized by higher iron demand and therefore remain more sensitive to the environment full of iron chelators (Feril Jr et al. 2017). Despite the proven effectiveness of EDTA on cell lines, the compound showed no antitumor efficiency in tumor-bearing mice (El-Naggar and El-Said 2020). Additionally, interesting results were obtained with the use of ferric-EDTA complex. In vivo studies on mice showed that increased dietary iron intake (in the form of EDTA-iron complex) increased the risk of colitis-associated colorectal cancer development. In the group of mice supplemented with a double dose of iron, on day 17, 15 out of 16 mice had histopathological confirmation of the tumor. In contrast, in the normal diet control group, colorectal tumors were found in 6 mice (Seril et al. 2002). Interestingly, mice with DSS-inducted colitis with intraperitoneal administration of iron did not show an enhanced risk of colon carcinogenesis compared to the control group. The results were contrary in the group of mice treated with oral iron supplementation (iron-EDTA) in a double dose compared to the standard diet. In the group tumor incidence was significantly increased. Besides, enhanced expression of iNOS and COX-2 proteins has been observed (Seril et al. 2005).

The differences between two supplements might arise from the differences in the composition of their water solutions. To show the concentration of biologically active free ferric ions and stable complex compounds, calculations were performed. Data used to describe the solutions with two analyzed compounds are described in Table 1.

Standard in vitro experiments happen under pH = 7.4 and 25 °C temperature. However, the oral administration of the ferric citrate and ferric-EDTA implies the contact of the complex compounds with the acidic environment of the stomach (pH ~ 3) and duodenum (pH ~ 6). The stability constants (Table 1) are not affected by pH, but the free Fe^{3+} hydrolyses, thus decreasing the form of complexed iron in the solution (Atkins and De Paula 2010). Total concentration of iron can be described by the molar fraction formulas:

$$C_{\mathrm{Fe}} = \left[\mathrm{Fe}^{3+}\right] + \left[\mathrm{FeOH}^{2+}\right] + \left[\mathrm{Fe(OH)}_2^{+}\right] + \left[\mathrm{Fe(OH)}_{3(\mathrm{aq})}\right] + \left[\mathrm{Fe(OH)}_4^{-}\right] + \left[\mathrm{complex}\right]$$

$$C_{\mathrm{Fe}} = \left[\mathrm{Fe}^{3+}\right] + \left[\mathrm{FeOH}^{2+}\right] + \left[\mathrm{Fe(OH)}_2^{+}\right] + \left[\mathrm{Fe(OH)}_{3(\mathrm{aq})}\right] + \left[\mathrm{Fe(OH)}_4^{-}\right] + \left[\mathrm{complex}\right]$$

Table 1 Summary of complex stability constants and hydrolysis constants of the components of the complexes

	Fe^{3+} Hydrolysis	Fe^{3+}—Citrate stability	Fe^{3+}—Edta stability
REACTIONS	$Fe^{3+} + H_2O \rightarrow Fe(OH)^{2+} + H^+$ $Fe(OH)^{2+} + H_2O \rightarrow Fe(OH)_2^+ + H^+$ $Fe(OH)_2^+ + H_2O \rightarrow Fe(OH)_{3(aq)} + H^+$ $Fe(OH)_{3(aq)} + H_2O \rightarrow Fe(OH)_4^- + H^+$	$Fe^{3+} + \text{citrate} \rightarrow Fe^{3+} - \text{citrate}$	$Fe^{3+} + EDTA \rightarrow Fe^{3+} - EDTA$
COMPLEX STABILITY CONSTANTS	$\log(\beta 1) = -2.19$, $\log(\beta 2) = -5.76$, $\log(\beta 3) = -14.3$, $\log(\beta 4) = -21.71$	$\beta_{\mathrm{Fe3+citrate}} = \frac{[Fe^{3+}-\text{citrate}]}{[Fe^{3+}][\text{citrate}]} = 10^{11.85}$	$\beta_{\mathrm{Fe3+-EDTA}} = \frac{[Fe^{3+}-\text{EDTA}]}{[Fe^{3+}][\text{EDTA}]} = 10^{25.10}$
ACID HYDROLYSIS CONSTANTS		$pK_{a1} = 3.13$, $pK_{a2} = 4.76$, $pK_{a3} = 6.4$	$pK_{a1} = 2$, $pK_{a2} = 2.7$, $pK_{a3} = 6.16$, $pK_{a4} = 10.26$
REFERENCES	(Stefánsson 2007)	(Silva et al. 2009)	(Gomathi 2000; Königsberger et al. 2000; Oades 2010)

Due to the fact that ligand-to-metal ratio remains 1:1 in the analyzed compounds, the above formulas are equal to each other:

$$C_{Fe} = C_{ligand}$$

Combining the formulas with the equations for stability and hydrolysis constants:

$$C_{Fe} = \left[Fe^{3+}\right] + \frac{\left[Fe^{3+}\right] \times \beta_1}{\left[H^+\right]} + \frac{\left[Fe^{3+}\right] \times \beta_2}{\left[H^+\right]^2} + \frac{\left[Fe^{3+}\right] \times \beta_3}{\left[H^+\right]^3} + \frac{\left[Fe^{3+}\right] \times \beta_4}{\left[H^+\right]^4} + \left[complex\right]$$

Combining all together, the formula for the non-complexed iron concentration was derived:

$$0 = \frac{\beta_{complex}}{k} \times \left[Fe^{3+}\right]^2 + \left(1 + \frac{\beta_1}{\left[H^+\right]} + \frac{\beta_2}{\left[H^+\right]^2} + \frac{\beta_3}{\left[H^+\right]^3} + \frac{\beta_4}{\left[H^+\right]^4}\right) \times \left[Fe^{3+}\right] - C$$

where k for EDTA complex:

$$k = \frac{1 + \frac{[H^+]}{K_{a4}} + \frac{[H^+]^2}{K_{a4}K_{a3}} + \frac{[H^+]^3}{K_{a4}K_{a3}K_{a2}} + \frac{[H^+]^4}{K_{a4}K_{a3}K_{a2}K_{a1}}}{1 + \frac{\beta_1}{[H^+]} + \frac{\beta_2}{[H^+]^2} + \frac{\beta_3}{[H^+]^3} + \frac{\beta_4}{[H^+]^4}}$$

And k for citrate complex:

$$k = \frac{1 + \frac{[H^+]}{K_{a3}} + \frac{[H^+]^2}{K_{a3}K_{a2}} + \frac{[H^+]^3}{K_{a3}K_{a2}K_{a1}}}{1 + \frac{\beta_1}{[H^+]} + \frac{\beta_2}{[H^+]^2} + \frac{\beta_3}{[H^+]^3} + \frac{\beta_4}{[H^+]^4}}$$

The data obtained by solving the equations were plotted to analyze the general distribution of iron forms in the working solutions under different pH conditions (see Fig. 1).

Due to the differences in the stability constants, solutions of both analyzed compounds differ in the fraction of complexed and non-complexed iron forms (see Fig. 2). Iron (III) citrate solution is composed of non-complexed iron and free citrate ions forms (Table 2). Conversely, in the iron (III)-EDTA solution almost all iron (III) ions are complexed with EDTA. Therefore, both components of iron citrate remain separated to some extent, whereas the EDTA complex in the same physiological pH remains mostly stable and does not dissociate. With the increase in H^+ concentration, the dissociation of both complexes occurs; however in case of ferric-EDTA complex, the process does not provide as much free iron ions as the dissociation of less stable ferric citrate.

Presented data may be interesting in regard to novel anticancer therapy approaches toward cancer treatment with the use of iron complexes. By applying an unstable complex to the biological system, the compound acts as both of the separated compounds. However, if especially stable complexes are added to the cells or the organism, iron would not be accessible. Here the outcome of the therapy would probably be less beneficial and more restricted toward the exact EDTA-iron complex.

Clinical trials involving the iron complexes in oncology

In clinical settings, we can distinguish two approaches that evaluate the potential of iron to target cancer. Namely, iron depletion affects iron-dependent cellular processes, delivery of iron to trigger oxidative stress or induce ferroptosis and consequently eliminate tumor cells (Torti and Torti 2020).

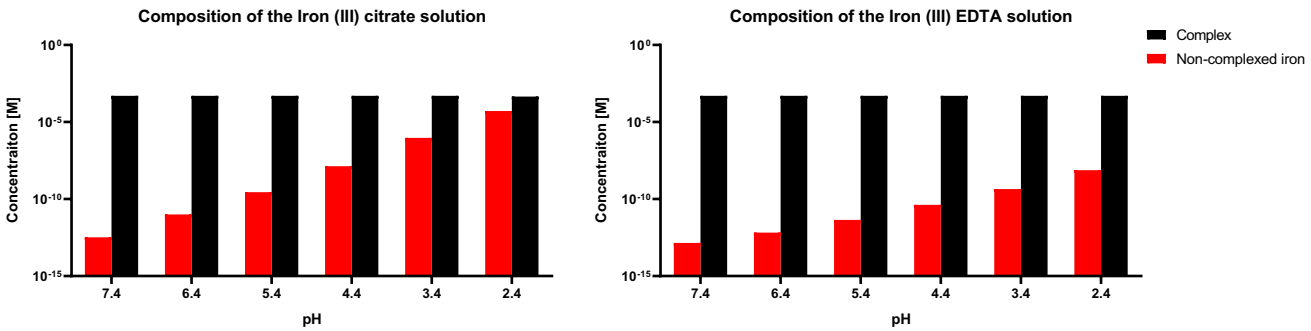

Fig. 2 The graphical representation of the concentration of complexed and non-complexed iron

Table 2 Summary of the iron chelators and the biological response toward treatment of cancer

Ligand	Cancer response	Reference
EDTA	Oxidative degeneration of 2-deoxyribose	(Schulman et al. 1995; Buss et al. 2005a, b)
CITRATE	Inhibition of the phosphofructokinase enzyme (cell is unable to perform glycolysis), inhibition of pyruvate dehydrogenase enzyme complex and succinate dehydrogenase enzyme of Krebs cycle—all of these actions force tumor cell to limit metabolism thereby inhibit proliferation	(Kantar et al. 2005; Halabe Bucay 2007)
DEFERASIROX	Decreasing cellular viability, inhibition of DNA replication and induction of its fragmentation in hepatoma cell lines	(Lui et al. 2013; Bedford et al. 2013)
	Polyamine synthesis reduction	
	Anti-neoplastic effect toward esophageal and lung tumor xenografts	
	Reduction of NF-κB activity in leukemia, prostate and breast cancer	
	Repressing signaling through mTOR pathway	
	Inhibition Wnt signaling by iron dependent mechanism	
	Antiproliferative features toward DMS-53 lung carcinoma cells	
DEFEROXAMINE	Suppressing thymidine incorporation of mitogen-stimulated lymphocytes	(Buss et al. 2005a; b)
	Inhibition of proliferation of the myeloid leukemia cell lines	
	DNA synthesis inhibition	
	Induction of apoptosis	
THIOSEMICARBAZONE	Inhibition of DNA synthesis	(de Siqueira et al. 2019)
	Stabilization of the complex between topoisomerase II and DNA through alkylation of thiol on the top II-DNA complex	
	Inhibition of ribonucleotide reductase by metal ion chelation	
	Inhibition of cathepsin L, which are overexpressed in some types of cancer—lung, prostate, gastrointestinal, breast. High levels of cathepsin L are associated with more aggressive tumors	
	Increase of metastasis suppressor NDRG1 which works on many of cellular signaling pathways like THF-β pathway, protein kinase	
	B/PI3K pathway. Higher levels of NDRG1 are negatively associated	
	with cancer growth and progression in such as prostate, breast, colon and pancreatic tumors	
CICLOPIROXAMINE	Suppression of cell proliferation in pancreatic cancer by reduction of Bcl-xL and surviving levels and activation of caspase 3 what took effect in higher levels of ROS leading all together to cell death	(Mihailidou et al.2018)
TACHYPYRIDINE	Toxicity toward breast tumor cells, bladder tumor cells and non-small lung tumor cells	(Buss et al. 2005a; b)
	Inhibition of ferritin synthesis in bladder cancer cells	
	Apoptosis induction in HeLa cells by induction of cleavage of caspase-9	
VLX600	Interference with intracellular iron metabolism by inhibiting electron transport chain, which leads to inhibition of ATP production and results in tumor cell death	(Mody et al. 2019)

Table 2 (continued)

Ligand	Cancer response	Reference
TRIAPINE	Inhibition of RR (ribonucleotide reductase) which is highly involved in DNA synthesis	(Buss et al. 2005a; b; Enyedy et al. 2010)
	Synergistic action with commonly used antitumor agents	
	Binding intracellular iron reduced and oxidized states and is potent In inducing cellular iron depletion	
DPC	Apoptosis induction in pancreatic cancer cells	(Kovacevic et al. 2011; Maqbool et al. 2020)
	Inhibition of pancreatic tumor growth, reducing tumor weight	
	Combination with tamoxifen reduced molecular pathways of proliferation of tamoxifen-resistant cells in estrogen receptor-positive breast cancer	
TALACTOFERRIN	Activation of lymphokine-activated killer cells and NK cells	(Hayes et al. 2006; Spadaro et al. 2007; Madan et al. 2013)
	Boosting the cytotoxicity of macrophages and polymorphonuclear cells	
	Enhancing levels of IL-6 and tumor necrosis factor-alfa in patients with IV stage non-small lung cancer	
	Inhibition of growth in squamous- (O12, SCC VII) and adeno- (TUBO, TSA) carcinomas in mice models	
	Immunostimulant in mice models – enhanced levels of IL-18 (small intestine and circulation), increased cytotoxicity of splenic NK cells, and increased CD4 + and CD8 + circulation	
FERUMOXYTOL	Stimulation of antitumor activity, synergistically with CpG in NSCLC with $EGFR^{L858R/T790M}$ mutation by enhanced expression of M1-like genes in macrophages (including tumor necrosis factor-α, IL-12, IL-1α, IL-1β, IL-6 and iNOS)	(Wang et al. 2019)
	Inhibition of proliferation and induction of apoptosis in FMT/CpG-pretreated macrophages supernatant of H1975 cells	
	Tumor growth suppression in mice (FMT/CpG)	
PRLX 93,936	Potent and selective activity against many tumor cell lines delivered from breast, colon, melanoma, lung, ovary, kidney and pancreas	(Sahasrabudhe et al. 2008)
	Activation of Ras pathway	
	Induction of caspase dependent apoptosis by affecting ion flux, cell cycle and polarization of mitochondrial membrane	

In clinical settings, both approaches are applied to enhance the sensitivity of cancer to chemotherapy and radiotherapy.

Among iron chelators, numerous clinical trials investigate Triapine as a potential drug against prostate cancer (NCT00054015), lung cancer (NCT00064064), breast cancer (NCT00095888) and pancreatic cancer (NCT00064051). The therapeutic potential of another iron chelator DpC (Dp4cycH4mT) is evaluated for advanced solid tumors (NCT02688101) (Guo et al. 2016).

Furthermore, gallium can be applied as an iron mimetic that dysregulates the iron-dependent proliferation of tumor cells (Chitambar 2012). Gallium nitrate underwent the clinical trial for brain tumors, neuroblastoma, rhabdomyosarcoma, non-Hodgkin's lymphoma, or refractory solid tumors (NCT00002543).

Studies show that lactoferrin the iron-binding glycoprotein has the potential to affect tumor iron homeostasis (Cutone et al. 2020). The recombinant human lactoferrin–talactoferrin entered clinical trials for renal cell carcinoma (NCT00095186) and lung cancer (NCT00923741) treatment.

The rationale of iron-based drugs application triggers the iron-based oxidative stress to eliminate tumor cells. Trujillo-Alonso et al. evaluated the application feasibility of ferumoxytol (Feraheme), a clinically approved iron

oxide nanoparticle used to treat iron deficiency, in AML treatment (Trujillo-alonso et al. 2019).

Interestingly popular ascorbate shows an anticancer effect based on iron-dependent oxidative stress (Cancer et al. 2017). Multiple trials evaluate its efficiency among others for treatment of lung cancer (NCT02420314), pancreatic cancer (NCT02905578), prostate cancer (NCT01080352), bladder cancer (NCT04046094), sarcoma (NCT04634227) and glioblastoma (NCT02344355).

Also, the anticancer effect of ferroptosis-inducing agents is based on triggering the excess iron in cancer therapy (Su et al. 2020). The studies on Erastin suggests the effectiveness of ferroptosis inducers as anticancer drugs (Zhang et al. 2020). One clinical trial investigates Erastin's analogue PRLX 93,936 for relapsed/refractory multiple myeloma (NCT01695590).

Table 2 summarizes the cancer response toward treatment with various iron complex compounds from both in vitro and in vivo studies.

Conclusions

Nowadays, anticancer research focuses on the development of novel and cytotoxic compounds. Ferric ions seem potent as the ferroptosis-inducing agents; thus, various studies concern the application of iron complex compounds in the anticancer therapy.

Iron-containing compounds, which are stable in the physiological conditions, act on cancer in a unique way. Namely, both components of the complex form an agent that interferes the metabolic pathways of the cells. Conversely, when the iron compound is not stable, the biological outcome depends on the interaction between both components of the complex. When the ligand poses a standalone cytotoxic effect on the cancer, the combination with ferric ions would be therapeutically beneficial. However, when the ligand stimulates the development and replication of the cell, the combination with ferric ions could even promote the progression of cancer.

Concluding, to obtain a good therapeutical potential of iron-containing drugs, the active compound should be characterized by (1) high stability of the complex and good standalone cytotoxicity or (2) low stability of the complex, but high cytotoxicity induced by the ligand that with the antiproliferative efficacy of ferric ions, would result in overall beneficial effect.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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