FEROPTOZA I NJEN KLINIČKI ZNAČAJ

PREGLEDNI RAD

REVIEW ARTICLE

FERROPTOSIS AND ITS CLINICAL SIGNIFICANCE

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SAŽETAK

Uvod: Feroptoza, koju su prvi put spomenuli Dikson i saradnici, 2012. godine, jeste put ćelijske smrti zavisan od gvožđa, uz neophodno prisustvo lipidnih peroksida. Mehanizam procesa i signalni putevi koji u njemu učestvuju se razlikuju u odnosu na ranije poznatu apoptozu, nekrozu i autofagiju. Dolazi i do zapaljenske reakcije, što ovaj put ćelijske smrti dodatno razlikuje od apoptotskog puta. Ferostatin dovodi do inhibicije ovog procesa.

Metode: Urađen je pregled literature dobijene pretraživanjem *Medline* baze podataka sa posebnim osvrtom na radove koji su se bavili značajem feroptoze u kliničkoj medicini, prvenstveno hematologiji.

Rezultati: Metabolizam gvožđa u malignoj i zdravoj ćeliji se razlikuje. Maligne ćelije dobro tolerišu oksidativni stres i izbegavaju feroptozu. Po literaturnim podacima, različiti ispitivani agensi stimulišu feroptozu i time postaju mogući terapijski agensi. Neki geni povezani sa metabolizmom gvožđa pokazali su prognostički značaj kod obolelih od difuznog B krupnoćelijskog limfoma.

Zaključak: Otkrivanje novih mehanizama ćelijske smrti i signalnih puteva koji su u taj proces uključeni dovodi do potencijalno nove ciljne terapije. Iako obećavaju, ovi rezultati zahtevaju validaciju kroz dalja istraživanja.

Ključne reči: gvožđe, limfom, terapijski agensi, ćelijska smrt

ABSTRACT

Introduction: Ferroptosis, mentioned as such for the first time in 2012 by Dixon et al., is an iron-dependent type of cell death that occurs in the presence of lipid peroxides. The mechanism of the process and the signaling pathways involved in it differ from the previously known apoptosis, necrosis, and autophagy. An inflammatory reaction also occurs, which further distinguishes this type of cell death from apoptosis. Ferrostatin inhibits this process.

Methods: A review of the literature obtained by searching the Medline database was performed, with a special focus on studies concerned with the importance of ferroptosis in clinical medicine, primarily hematology.

Results: Iron metabolism in malignant and healthy cells differs. Malignant cells tolerate oxidative stress well and avoid ferroptosis. According to literature data, various tested agents stimulate ferroptosis and thus become possible therapeutic agents. Some genes linked to iron metabolism have shown prognostic significance in patients with diffuse large B-cell lymphoma.

Conclusion: The discovery of new mechanisms of cell death and the signaling pathways involved in this process leads to potentially new target therapy. Although promising, these results require validation through further research.

Keywords: iron, lymphoma, therapeutic agent, cell death

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Primljeno • *Received*: August 4, 2024; **Revidirano • *Revised*:** September 20, 2024; **Prihvaćeno • *Accepted*:** September 22, 2024; ***Online first*:** September 25, 2024

DOI: 10.5937/smclk5-52576

UVOD

Feroptoza, prvi put pomenuta u radu Diksona i saradnika, 2012. godine, jeste neapoptotski, nenekrozni i neautofagni put ćelijske smrti [1]. Dolma i saradnici su, 2003. godine, otkrili da erastin može da izazove nov način ćelijske smrti, a potom su Jagoda i Jang objavili da helatori gvožđa inhibiraju ovaj proces u čijoj osnovi se nalazi disfunkcija mitohondrija [2-4].

Kao što i naziv sugeriše, feroptoza zavisi od gvožđa, dešava se u prisustvu lipidnih peroksida, a ferostatin je inhibira. Za razliku od apoptoze, u ovom procesu dolazi do privlačenja inflamatornih ćelija i reakcije zapaljenja. U procesu apoptoze, ćelijska membrana bubri i dolazi do kondenzacije hromozoma. U feroptozi se mitohondrije smanjuju, njihove membrane su hiperdenznije a mitohondrijalne kriste su manje ili ih nema [1,5-9]. Biohemijski posmatrano, koncentracija glutationa u ćeliji je manja. Glutation peroksidaza 4 (engl. *glutathione peroxidase – GPX4*) je enzim koji dovodi do redukcije lipidnih peroksida, a čija aktivnost je u ovom procesu takođe smanjena. Jon Fe^{2+} oksidiše lipide, koncentracija reaktivnih kiseoničnih radikala (engl. *reactive oxygen species – ROS*) raste i pokreće se feroptoza [5]. U ovom putu su važni i transmembranski kanali u ćelijskim membranama kroz koje se vrši razmena cistina i glutamata. Cistin ulazi u ćeliju i u njoj se redukuje u cistein, neophodan u sintezi glutationa [1,5]. Ovo je i mesto na kojem deluju induktori feroptoze. Helatori gvožđa inhibiraju ovaj proces, ali i ferostatin 1 (Fer-1), vitamin E i liprokstatin 1.

Feroptoza zavisi od gvožđa, te je očekivano da faktori koji učestvuju u metabolizmu gvožđa ili u regulaciji njegovog metabolizma imaju ili već dokazanu ili moguću ulogu u regulaciji ovog puta ćelijske smrti, čineći ćeliju manje ili više osetljivom na feroptozu [6,10,11].

Metabolizam gvožđa u zdravoj i malignoj ćeliji

Kada govorimo o regulaciji metabolizma gvožđa reč je o kontroli na ćelijskom nivou i sistemskoj regulaciji metabolizma gvožđa. Homeostaza gvožđa u ćeliji se reguliše uglavnom posttranskripcionom kontrolom gena putem interakcije elemenata i belančevina senzitivnih na koncentraciju gvožđa (engl. *iron-responsive elements – IRE*; *iron-responsive proteins – IRP*) [12].

Sistemska homeostaza je kontrolisana hepcidinom, jetrenom belančevinom čija se koncentracija, između ostalog, povećava u inflamaciji, pod dejstvom interleukina-6 i dovodi do internalizacije i degradacije feroportina. Feroportin je, po podacima iz dostupne literature u ovom momentu, jedini eksporter gvožđa iz ćelija [12].

Hranom uneseno gvožđe izlazi iz enterocita putem feroportina, u trovalentnom obliku, i vezan za transfer-

INTRODUCTION

Ferroptosis, first mentioned in a study by Dixon et al. in 2012, is a non-apoptotic, non-necrotic, and non-autophagic form of cell death [1]. In 2003, Dolma et al. discovered that erastin can cause a new type of cell death, upon which Yagoda and Yang reported that iron chelators inhibited this process, which is based on mitochondrial dysfunction [2-4].

As the name suggests, ferroptosis is iron-dependent, it occurs in the presence of lipid peroxides, and is inhibited by ferrostatin. Unlike apoptosis, this process involves the involvement of inflammatory cells and an inflammatory reaction. In the process of apoptosis, the cell membrane swells and chromosomes condense. In ferroptosis, mitochondria are reduced, their membranes are hyperdense, and mitochondrial cristae are smaller or absent [1,5-9]. In terms of biochemistry, the concentration of glutathione in the cell is lower. Glutathione peroxidase 4 (GPX4) is an enzyme that causes the reduction of lipid peroxides, and whose activity is also reduced in this process. The Fe^{2+} ion oxidizes lipids, the concentration of reactive oxygen species (ROS) increases, and ferroptosis is triggered [5]. Transmembrane channels in cell membranes through which cystine and glutamate are exchanged are also important in this process. Cystine enters the cell and is reduced to cysteine, which is necessary for the synthesis of glutathione [1,5]. This is also the site where ferroptosis inducers act. Iron chelators inhibit this process, but so do ferrostatin 1 (Fer-1), vitamin E, and liproxstatin 1.

Ferroptosis depends on iron, which is why it can be expected that factors involved in iron metabolism or in the regulation of its metabolism have either already proven or potential roles in the regulation of this type of cell death, making the cell more or less sensitive to ferroptosis [6,10,11].

Iron metabolism in healthy and malignant cells

When the regulation of iron metabolism is concerned, we speak of control at the cellular level and of systemic iron metabolism regulation. Iron homeostasis in the cell is regulated mainly by post-transcriptional control of genes via the interaction of iron-responsive elements (IRE) and iron-responsive proteins (IRP) [12].

Systemic homeostasis is controlled by hepcidin, a liver protein whose concentration, among other things, increases in inflammation, under the influence of interleukin-6, leading to the internalization and degradation of ferroportin. According to currently available literature data, ferroportin is the only exporter of iron from cells [12].

Dietary iron leaves the enterocytes via ferroportin, as trivalent iron, and is transported to tissues bound

in se prenosi do tkiva. Kompleks gvožđe-transferin se vezuje za transferinski receptor 1 (TfR1) i procesom endocitoze ulazi u ćelije. U ćeliji prelazi iz trovalentnog u dvovalentni oblik i ugrađuje se u različite enzime, ulazi u sastav hema, učestvuje u oksidativnoj fosforilaciji i sintezi dezoksiribonukleotida. Suvišno gvožđe se skladišti u feritinu.

Pokazalo se da dve belančevine – ZIP 8 i ZIP 14, s poznatom ulogom u prenošenju cinka, učestvuju i u transportu gvožđa koje nije vezano za transferin (engl. *non-transferrin-bound iron – NTBI*) kroz ćelijsku membranu [12-16], a imaju značaja i u hemohromatozi [16]. Njihovo mesto u drugim stanjima s nakupljanjem gvožđa, poput maligniteta i nekih anemija, ostaje nejasno [12]. Proteini *PCBP1* i *PCBP2* (engl. *poly(rC) binding protein 1*; *poly(rC) binding protein 2*) su šaperoni (engl. *chaperones*) – oni prate dvovalentno gvožđe u citoplazmi i imaju ulogu u njegovoj distribuciji u ćeliji [12,17,18], dok koaktivator jedarnog receptora 4 (engl. *nuclear receptor coactivator 4 – NCOA4*) učestvuje u autofagiji feritina, te tako deluje na iskoristivost gvožđa koje je u njemu uskladišteno [12,19-22].

Gvožđe koje se nalazi u ćeliji deponovano u feritinu je netoksično ali je, dok je na taj način vezano, nedostupno za ćelijske potrebe. Da bi bilo iskoristivo, mora se osloboditi, obično degradacijom u lizozomima [19-22]. Izgleda da je način degradacije feritina isti i u stanjima s nakupljanjem gvožđa, a i kad postoji njegov nedostatak, ali se lizozomima isporučuje na drukčiji način [12,20]. Kad postoji deficit gvožđa, dolazi do autofagije, a kad postoji nakupljanje gvožđa, aktivni su drugi putevi [20]. *NCOA4* je bitan činilac u autofagiji feritina kod nedostatka gvožđa i za regulaciju homeostaze gvožđa u slezini [23,24]. Ako nema nedostatka gvožđa, neautofagni putevi razgradnje feritina su manje aktivni u malignim ćelijama nego u zdravim, te su zato i maligne ćelije otpornije na oštećenja koje izaziva višak gvožđa [20]. Reaktivacija tih puteva bi mogla povećati toksičnost gvožđa za neoplastičnu ćeliju i možda biti nova terapijska strategija [12].

Ćelije kancera menjanju metabolizam tiola, koji su intraćelijski redoks puferi, a oksidativni stres zbog viška gvožđa povezan je sa karcinogenezom [25]. Već smo spomenuli da maligne ćelije zadržavaju veću količinu gvožđa, u lizozomima, ali i u citoplazmi i mitohondrijama. To dovodi do poremećaja njihove funkcije, preuzima se više glukoze i u prisustvu kiseonika, u procesu aerobne glikolize, produkuju se laktati. Ovaj fenomen je poznat kao Varburgov efekat. Osobina većeg preuzimanja glukoze od strane malignih ćelija koristi se u primeni pozitronske emisione tomografije (PET) u dijagnostici i proceni terapijskog efekta kod bolesnika sa malignim oboljenjima [26].

to transferrin. The iron-transferrin complex binds to the transferrin receptor 1 (TfR1) and enters cells via endocytosis. In the cell, trivalent iron is transformed into divalent iron and, as such, it is incorporated into various enzymes, it enters the composition of heme, and participates in oxidative phosphorylation and synthesis of deoxyribonucleotides. Excess iron is stored in ferritin.

It has been shown that two proteins – ZIP 8 and ZIP 14, with a known role in zinc transport, also participate in the transport of non-transferrin-bound iron (NTBI) through the cell membrane [12-16], and they are also important in hemochromatosis [16]. Their role in other conditions characterized by iron overload, such as malignancies and some forms of anemia, remains unclear [12]. Poly(rC) binding protein 1 (PCBP1) and poly(rC) binding protein (PCBP2) are iron chaperones – they accompany divalent iron in the cytoplasm and play a role in its distribution in the cell [12,17,18], while nuclear receptor coactivator 4 (NCOA4) participates in the autophagy of ferritin, thus affecting the utilization of iron stored in it [12,19-22].

The iron found in the cell deposited in ferritin is non-toxic, however, while bound in such a way, it is unavailable for cellular needs. For it to be usable, it must be released, usually by degradation in the lysosomes [19-22]. It seems that the method of ferritin degradation is the same in conditions characterized by iron overload and those characterized by iron deficiency, however, it is delivered to lysosomes differently [12,20]. When there is iron deficiency, autophagy occurs, and when there is iron overload, other pathways are active [20]. NCOA4 is an essential factor in ferritin autophagy in iron deficiency, as well as in the regulation of iron homeostasis in the spleen [23,24]. If there is no iron deficiency, non-autophagic pathways of ferritin degradation are less active in malignant cells than in healthy ones, and therefore malignant cells are more resistant to damage caused by excess iron [20]. The reactivation of these pathways could increase the toxicity of iron to the neoplastic cell and potentially be a new therapeutic strategy [12].

Cancer cells alter the metabolism of thiols, which are intracellular redox buffers, and oxidative stress, due to excess iron, is associated with carcinogenesis [25]. We have already mentioned that malignant cells retain excess iron in lysosomes, but also in the cytoplasm and mitochondria. This leads to the disruption of their function, the uptake of glucose is increased, and, in the presence of oxygen, in the process of aerobic glycolysis, lactates are produced. This phenomenon is known as the Warburg effect. The property of higher uptake of glucose by malignant cells is used in the application of positron emission tomography (PET) in the diagnostics and assessment of the therapeutic effect of treatment in patients with malignant diseases [26].

Agensi koji utiču na feroptozu

Iz svega prethodno navedenog, vidi se da maligna ćelija, bez obzira na stalnu izloženost oksidativnom stresu, ima sposobnost da ga toleriše i izbegne feroptozu [25]. Erastin i sulfasalazin blokiraju ulazak cistina kroz kanale u fosfolipidnom delu ćelijske membrane. Na taj način, kao i drugi agensi koji utiču na smanjenje produkcije glutationa u eksperimentalnim uslovima, dovode do supresije tumorskog rasta i feroptoze [25,27-29]. Testiranja su pokazala da promena metabolizma cistina i glutationa dovodi do povišene koncentracije slobodnih kiseoničnih radikala, a antioksidativna terapija prevenira ćelijsku smrt [10]. Erastin je jak induktor feroptoze koji inhibira transmembranske antiportere za razmenu intraćelijskog glutamata i ekstraćelijskog cistina. Glutation učestvuje u održavanju redoks ravnoteže i, kad ga nema u dovoljnoj koncentraciji, nagomilavaju se slobodni kiseonični radikali, a već je rečeno da je za njegovu sintezu neophodan ulazak cistina u ćeliju [30-32]. Dikson i saradnici navode da sulfasalazin i sorafenib blokiraju ovaj antiporter i dovode do selektivne feroptoze, s tim da je, u ovoj studiji, erastin 2.500 puta snažnije inhibirao ovaj sistem od sulfasalazina. Dodatni pokazatelj značaja gvožđa u feroptozi je i to što deferoksamin, koji je helator gvožđa, suprimira ćelijsku smrt indukovanu erastinom, dok je dodatni unos gvožđa potencira [1,27,33]. Ispitivanja na kulturi ćelija difuznog B krupnoćelijskog limfoma su pokazala značajnu osetljivost na ćelijsku smrt indukovanu erastinom [34]. U drugom istraživanju je pokazano da erastin povećava senzitivnost leukemijskih ćelija akutne limfocitne leukemije (ALL) na efekat *SMAC* mimetika *LCL-161* (engl. *second mitochondria-derived activator of caspases – SMAC mimetic LCL-161*) i da sinergističkim delovanjem dolazi do smrti leukemijske ćelije [35]. Drugi autori navode dozno zavisan efekat erastina na ćelije akutne mijeloidne leukemije, jer je u njihovim istraživanjima na kulturi ćelija dovodio do supresije njihovog rasta, ali i mešanog tipa ćelijske smrti. Supresija ovog efekta se ostvarila ferostatinom 1, inhibitorom feroptoze ili nekrostatinom 1, inhibitorom nekroptoze, ali ne i inhibitorom kaspaznog puta, koji deluje na apoptozu, niti hlorohinom, koji inhibiše autofagiju. Na kulturi ćelija hronične mijeloidne leukemije, akutne T limfoblastne leukemije i akutne promijelocitne leukemije, ovi efekti nisu postignuti. Ćelije akutne mijeloidne leukemije (AML) su, uz male doze erastina, bile značajno osetljivije na citozin arabinozid i antracikline [36], uz napomenu da je upotrebljen doksorubicin, a ne daunoblastin. U nedavno objavljenom radu, druga grupa autora je na *in vitro* modelu pokazala erastinom povećanu senzitivnost ćelija akutne mijeloidne leukemije na venetoklaks [37]. Fibroblasti i dendritske ćelije

Agents affecting ferroptosis

Based on all the above, it is evident that malignant cells, regardless of constant exposure to oxidative stress, can tolerate it and avoid ferroptosis [25]. Erastin and sulfasalazine block the entry of cystine through channels in the phospholipid part of the cell membrane. In this way, just like other agents affecting the reduced production of glutathione in experimental conditions, they lead to tumor growth suppression and ferroptosis [25,27-29]. Tests have shown that change in the metabolism of cystine and glutathione leads to an increased concentration of free oxygen radicals, while antioxidant therapy prevents cell death [10]. Erastin is a strong inducer of ferroptosis that inhibits transmembrane antiporters for intracellular glutamate and extracellular cystine exchange. Glutathione participates in maintaining the redox balance and when its concentration is insufficient, free oxygen radicals accumulate, and it has already been said that cystine entry into the cell is necessary for its synthesis [30-32]. In their study, Dixon et al. reported that sulfasalazine and sorafenib blocked this antiporter leading to selective ferroptosis, however, erastin inhibited this system 2,500 times more potently than sulfasalazine. An additional indicator of the importance of iron in ferroptosis is that deferoxamine, an iron chelator, suppresses erastin-induced cell death, while additional iron intake promotes it [1,27,33]. Studies on diffuse large B-cell lymphoma cell cultures have shown significant sensitivity to erastin-induced cell death [34]. In another study, it was shown that erastin increased the sensitivity of leukemic cells in acute lymphocytic leukemia (ALL) to the effect of the second mitochondria-derived activator of caspases (SMAC) mimetic LCL-161 and that the synergistic action led to the death of leukemic cells. [35]. Other authors have reported a dose-dependent effect of erastin on acute myeloid leukemia cells, since, in their studies of cell cultures, it led to the suppression of their growth, but also to a mixed type of cell death. The suppression of this effect was achieved by ferrostatin 1, an inhibitor of ferroptosis, or necrostatin 1, an inhibitor of necroptosis, but not by an inhibitor of the caspase pathway, which acts on apoptosis, nor by chloroquine, which inhibits autophagy. These effects were not achieved in chronic myeloid leukemia, acute T lymphoblastic leukemia, and acute promyelocytic leukemia cell cultures. Acute myeloid leukemia (AML) cells were significantly more sensitive to cytosine arabinoside and anthracyclines [36] when low doses of erastin were administered, where doxorubicin and not daunoblastin was used. In a recent study, another group of authors demonstrated, in an in vitro model, that erastin increased the sensitivity of acute myeloid leukemia cells to venetoclax [37]. Fibroblasts and dendritic cells make cystine, which lymp-

stvaraju cistin od kojeg zavise limfoidne ćelije, jer moraju da ga unesu da bi sintetisale cistein. Pokazano je da sulfasalazin, delujući na sistem kanala kroz koji se cistin transportuje u kulturi limfomskih ćelija, dovodi do prekida njihove proliferacije, a kasnije je na životinjskom modelu sa transplantiranim limfomom potvrđena supresija tumorskog rasta; za organizam domaćina nije bilo veće štetnosti [38]. Indukcija feroptoze je postignuta na kulturi ćelija 60 tumorskih linija delovanjem derivata artemisinina. Geni odgovorni za kodiranje belančevina koje učestvuju u metabolizmu gvožđa, poput transferina, transferinskih receptora 1 i 2, označeni su kao prediktori osetljivosti na artemisinin [39].

Feroptoza u krupnoćelijskom B limfomu

Krupnoćelijski B limfom (engl. *large B-cell lymphoma – LBCL*) čini oko trećinu svih ne-Hočkinovih limfoma, što znači da predstavlja najčešću limfoproliferativnu neoplazmu kod odraslih pacijenata [40]. Prvom terapijskom linijom, odnosno imunohemioterapijom po *R-CHOP* (rituksimab-ciklofosfamid, doksorubicin, vinkristin, prednizolon) protokolu, kompletna remisija se postiže u oko 60% bolesnika, ali lečenje pacijenata sa rezistentnom ili relapsirajućom (R/R) bolešću je i dalje klinički problem. Nisu svi bolesnici sa R/R *LBCL* podobni za autolognu transplantaciju matičnih ćelija, niti je *CAR* T ćelijska terapija (engl. *chimeric antigen receptors* (*CAR*) *T-cell therapy*) široko dostupna. Bispecifična antitela, u kombinaciji s drugim lekovima, uglavnom su dostupna kroz kliničke studije, a često se desi da bolesnici sa R/R bolešću ne ispunjavaju kriterijume za uključivanje u studiju ili su studije nedostupne. Široko primenjivani standardni skorovi rizika su prognostički alati kreirani da procene šansu za preživljavanje pre i u eri lečenja rituksimabom [41,42]. Uz to, *LBCL* je heterogena bolest koja je i u novoj klasifikaciji Svetske zdravstvene organizacije (SZO) predstavljena sa više entiteta [43], gde u tumačenjima nekad postoje preklapanja i različito viđenje izazova, iz ugla patologa i kliničara [44]. Postoji potreba za razvojem novih prognostičkih skorova, ali i terapeutika koji bi delovali drugim mehanizmima.

Gvožđe je neophodno za mnoge procese, uključujući i ćelijsku proliferaciju [45,46]. Devin i saradnici su razvili novi skor, *IS* (engl. *iron score*) zasnovan na profilu ekspresije gena odgovornih za metabolizam gvožđa. U dve nezavisne kohorte pacijenata sa *LBCL*, koje su obuhvatile 233 i 181 ispitanika, 11 gena je pokazalo prognostički značaj. Visoka ekspresija osam gena je definisala grupu sa lošom prognozom. *IS* je dodatno validiran u tri kohorte u odnosu na ukupno preživljavanje (engl. *overall survival – OS*) [47]. *IS* je bio značajno viši u grupi *LBCL* porekla aktivisanih B limfocita (engl. *activated B-cell – ABC*) u odnosu na one porekla germi-

hoid cells depend on because they must absorb it in order to synthesize cystine. It has been demonstrated that sulfasalazine, acting on the system of channels through which cystine is transported in a culture of lymphoma cells, leads to the interruption of their proliferation, and later the suppression of tumor growth was confirmed in an animal model with transplanted lymphoma; there was no major harm to the host organism [38]. Induction of ferroptosis was achieved on a cell culture of 60 tumor lines by the action of artemisinin derivatives. Genes responsible for coding proteins involved in iron metabolism, such as transferrin, transferrin receptors 1 and 2, have been identified as predictors of sensitivity to artemisinin [39].

Ferroptosis in large B-cell lymphoma

Large B-cell lymphoma (LBCL) accounts for about a third of all non-Hodgkin's lymphomas, which makes it the most common lymphoproliferative neoplasm in adult patients [40]. With first-line treatment, i.e. immunochemotherapy with the R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, prednisolone) regimen, complete remission is achieved in about 60% of patients, however, the treatment of patients with resistant or relapsing (R/R) disease remains a clinical problem. Not all patients with R/R LBCL are eligible for autologous stem cell transplantation, nor is chimeric antigen receptors (CAR) T-cell therapy widely available. Bispecific antibodies, combined with other drugs, are mostly available through clinical studies, however, it often happens that patients with R/R disease do not meet the criteria for inclusion in a study or that the studies are unavailable. Widely used standard risk scores are prognostic tools designed to estimate the probability of survival before and during the rituximab treatment era [41,42]. Furthermore, LBCL is a heterogeneous disease represented by multiple entities in the new World Health Organization (WHO) classification [43], where there are sometimes overlapping interpretations and different views of the challenges, from the point of view of pathologists and clinicians [44]. New prognostic scores, as well as therapeutic agents that would act via other mechanisms, need to be developed.

Iron is necessary for many processes, including cell proliferation [45,46]. Devin et al. developed a new score – iron score (IS), based on the expression profile of genes responsible for iron metabolism. In two independent cohorts of LBCL patients, which included 233 and 181 subjects, 11 genes showed prognostic significance. High expression of eight genes defined the poor prognosis group. IS was additionally validated in three cohorts in relation to overall survival (OS) [47]. IS was significantly higher in the activated B-cell-

nativnog centra (engl. *germinal-center B-cell-like – GCB*) [47]. Isti autori su na ćelijskim linijama *LBCL*, odvojeno *ABC* i *GCB* tipa, testirali efekat helatora gvožđa, deferasiroksa i deferoksamina, na vijabilnost limfomskih ćelija i videli, u zavisnosti od koncentracije, smanjenu vijabilnost ćelija nakon tretmana helatorima gvožđa. U istom radu je testiran i terapijski efekat irinomicina i ustanovljeno da u nanomolarnim koncentracijama inhibira rast tumorskih ćelija. Suplementacija gvožđem je inhibirala efekat helatora na limfomske ćelije, ali ne i efekat irinomicina, što je sugerisalo drukčiji mehanizam dejstva. Naime, helatori gvožđa su dovodili do apoptoze limfomskih ćelija a irinomicin je dovodio do sekvestracije gvožđa u lizozomima, deplecije gvožđa u ćelijskom citosolu i degradacije feroportina autofagijom u lizozomima, te produkcije reaktivnih kiseoničnih radikala koji su vodili ka feroptozi [47].

Šmit i saradnici, u radu objavljenom 2021. godine, pokazuju da dimetilfumarat, lek odobren u terapiji multiple skleroze i psorijaze, izazivajući feroptozu i slabeći *NF-kB/STAT 3* signalni put deluje na *LBCL* ćelije i *ABC* i *GCB* tipa [48].

Grupa naučnika iz Kine je, pre godinu dana, objavila da je povišen nivo glutamina povezan sa lošijim ishodom kod obolelih od *LBCL*, a da je koncentracija derivata glutamina, αketo-glutarata, obrnuto korelirala sa agresivnošću *LBCL* [49]. Akumulacija αketo-glutarata je dovodila do oksidativnog stresa u dabl hit limfomu (engl. *double-hit lymphoma – DHT*). Visok nivo slobodnih kiseoničnih radikala izazivao je akumulaciju lipidnih peroksida, došlo je do aktivacije TP53 i feroptoze [49].

ZAKLJUČAK

Sve je više radova koji se bave feroptozom u različitim malignitetima, pa i hematološkim. Ispituju se potencijalno nove uloge već poznatih lekova i mogućnosti njihove šire primene i sinergističkog dejstva sa standardnom terapijom. Otkrivanje novih mehanizama ćelijske smrti i signalnih puteva koji su u taj proces uključeni dovodi do otkrivanja potencijalno nove ciljne terapije. Sve ovo iz ugla kliničara deluje vrlo interesantno i ohrabrujuće, ali su neophodne dalje kliničke studije i validacija rezultata dobijenih *in vitro* i na laboratorijskim životinjama.

NAPOMENA: Osnove metabolizma gvožđa i feroptoze su ispitivane u okviru doktorske disertacije prvog autora, odbranjene 2022. godine.

Sukob interesa: Nije prijavljen.

like (ABC) LBCL group, as compared to the germinal-center B-cell-like (GCB) LBCL group [47]. The same authors tested the effect of iron chelators deferasirox and deferoxamine on the viability of lymphoma cells on LBCL cell lines (separately ABC and GCB types) and, depending on the concentration, observed reduced cell viability after treatment with iron chelators. In the same study, the therapeutic effect of irinomycin was tested. It was found that nanomolar concentrations of irinomycin inhibited tumor cell growth. Iron supplementation inhibited the effect of chelators on lymphoma cells, but not the effect of irinomycin, suggesting a different action mechanism. Namely, iron chelators led to lymphoma cell apoptosis, while irinomycin led to iron sequestration in lysosomes, iron depletion in the cell cytosol, ferroportin degradation by autophagy in lysosomes, and the production of reactive oxygen radicals leading to ferroptosis [47].

In a study from 2021, Schmidt et al. showed that dimethyl fumarate, a drug approved for the treatment of multiple sclerosis and psoriasis, acted on both ABC LBCL and GCB LBCL cells by causing ferroptosis and weakening the NF-kB/STAT 3 signaling pathway, [48].

A year ago, a group of scientists from China announced that an elevated level of glutamine was associated with a poorer outcome in LBCL patients, and that the concentration of the glutamine derivative, αketo-glutarate, was inversely correlated with the aggressiveness of LBCL [49]. Accumulation of α-keto-glutarate led to oxidative stress in double-hit lymphoma (DHT). A high level of free oxygen radicals caused the accumulation of lipid peroxides, leading to the activation of TP53 and to ferroptosis [49].

CONCLUSION

There is an increasing number of studies dealing with ferroptosis in various malignancies, including hematological malignancies. The potential new roles of already-known drugs and the possibilities of their wider application and synergistic effect with standard therapy are being examined. The discovery of new cell death mechanisms and the signaling pathways involved in that process leads to the discovery of potentially new target therapies. From the clinician's point of view, all this seems very interesting and promising, however, further clinical studies and validation of the results obtained in vitro and on laboratory animals are necessary.

NOTE: The basic principles of iron metabolism and ferroptosis were investigated in the doctoral dissertation of the first author of this study. The thesis was defended in 2022.

Conflict of interest: None declared.

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