Molekularni utjecaj glutation-peroksidaza u antioksidacijskim procesima

Molecular impact of glutathione peroxidases in antioxidant processes

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Sažetak

Reaktivni radikali kisika (ROS) stvaraju se tijekom različitih patoloških procesa u povećanim koncentracijama. Oni su uzrok peroksidacije lipida i oksidacije DNA i proteina zbog svoje visoke kemijske reaktivnosti. Međutim, mehanizmi antioksidacijske zaštite, uključujući različite antioksidacijske enzime, sprječavaju oštećenja tkiva i druge komplikacije povezane s ROS. Ovaj je pregled usredotočen na učinke različitih glutation-peroksidaza (GPX) na molekularnu kontrolu toksikološkog djelovanja reaktivnih kisikovih radikala. Nadalje, opisuju se specifična biokemijska svojstva, sinteza i uloga svakog izoenzima glutation-peroksidaze u biološkim procesima. Male molekule koje djeluju kao oponašatelji aktivnog mjesta glutation-peroksidaza mogle bi postati novo sredstvo u liječenju mnogih oboljenja.

Ključne riječi: antioksidacijski proces, glutation-peroksidaza, motiv SECIS, glutation, selen, izoenzimi

Abstract

Reactive oxygen species (ROS) are produced during different pathological processes in increased concentrations. They cause lipid peroxidation and oxidation of DNA and proteins due to their high chemical reactivity. However, antioxidative defense mechanisms, including different antioxidant enzymes, prevent tissue damages and other ROS-related complications. The focus of this review is on effects of different glutathione peroxidases (GPXs) on molecular control of reactive oxygen species toxicology. Furthermore, specific biochemical properties, synthesis and role of each glutathione peroxidase isoenzyme in biological processes are described. Small molecules acting as mimetics of the active site of glutathione peroxidases could become new tools for treatment of many diseases.

Key words: antioxidant process, glutathione peroxidase, SECIS motif, glutathione, selenium, isoenzymes

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Uvod

Proteklih je 20 godina pridana velika pozornost ulozi oksidacijskog stresa kojeg uzrokuju reaktivni radikali kisika (engl. *reactive oxygen species*, ROS) te razlikama u antioksidacijskim mehanizmima između pojedinaca, posebice kod bolesti koje ovise o dobi kao što su karcinom, artritis, ateroskleroza, neurodegenerativni poremećaji i dr. (1,2).

Antioksidacijska zaštita je važna u uklanjanju slobodnih radikala jer osigurava maksimalnu zaštitu bioloških mjesta kao što su tiolne skupine koje su dio aktivnih mjesta u nekim metabolizirajućim enzimima (2,3). Dobar antioksidans specifično potiskuje slobodne radikale, kelira redoks-metale, međusobno djeluje s drugim antioksidansima unutar antioksidacijske mreže, ima povoljan učinak na

Introduction

In the past 20 years great stress has been laid on the role of oxidative stress caused by reactive oxygen species (ROS), differences in antioxidant mechanisms among individuals, especially in age-dependent diseases such as cancer, arthritis, arteriosclerosis, neurodegenerative disorders and others (1,2).

Antioxidant defense is important in the removal of free radicals, providing the maximal protection of biological sites such as thiol groups which are part of active sites in some metabolizing enzymes (2,3). A good antioxidant should specifically quench free radicals, chelate redox metals, interact with other antioxidants within an antioxidant network, have a positive effect on gene expression,

izražaj gena, lako se apsorbira, ima fiziološki relevantnu koncentraciju u tkivima i biološkim tekućinama, te djeluje i u vodenim i/ili membranskim domenama (2). Najdjelotvorniji enzimski antioksidansi obuhvaćaju superoksid-dismutazu, katalazu i glutation-peroksidazu. Neenzimski antioksidansi uključuju tiolne antioksidanse (glutation, tioredoksin i lipoičnu kiselinu), vitamin C, vitamin E, karotenoide, prirodne flavonoide, te druge spojeve (selen) (4). Ovaj je pregled usredotočen na skupinu enzima, tj. glutation-peroksidaze (GPX), koji predstavljaju glavne enzime u mehanizmu antioksidacijske zaštite ovisnom o glutationu. Specifična biokemijska obilježja, sinteza i uloga svakog izoenzima GPX opisana su u biološkim procesima. Identificirano je barem 7 vrsta GPX (5,6) i njihova su obilježja prikupljena u tablici podijeljenoj na sljedeće vrste: citosolne (cGPX ili GPX1), gastrointestinalne (GI-GPX ili GPX2), plazmatske (pGPX ili GPX3), fosfolipid-hidroperoksidne (PHGPX ili GPX4), te glutation-peroksidaze GPX5 i GPX6. Nadalje, dan je i prikaz neenzimskih antioksidansa uključenih u aktivnost GPX.

Biosinteza glutation-peroksidaza

Biosinteza glutation-peroksidaza je slična biosintezi svih selenoproteina koja ovisi o dostupnosti selena (Se). Godine 1973. utvrđeno je da je Se strukturna sastavnica aktivnog središta životinjskog enzima stanične glutation-peroksidaze (GPX1) (6,7). Otada je identificirano 30 novih selenoproteina, od kojih je 15 pročišćeno te im je karakterizirana biološka funkcija (7-9).
Selen se kao selenocistein (Sec) ugrađuje u aktivno mjesto rastućeg višepeptidnog lanca kojeg kodira UGA. Kotranslacijska ugradnja Sec u selenoproteine uzrokuje znatne probleme stanici koja mora prepoznati UGA kao Sec-kodon, a ne kao translacijski STOP-signal (10-12).
Kloniranje GPX1 je dovelo do identifikacije specifičnog eukariotskog Sec-insercijskog sekvencijskog (SECIS) elementa kao strukture s ukosnicom smještene na 3' neprevedenim regijama (UTR) mRNA glutation-peroksidaze. Element SECIS, koji predstavlja aktivnu točku, jest signal koji iznova kodira UGA kao dio okvira od Stop-signala do Sec-kodona (13). Takav složeni slijed selenu očito pruža mnogo mogućnosti za posttranskripcijsku regulaciju biosinteze selenoproteina. Međutim, pomanjkanje selena rezultira preranim dovršetkom lanca na kodonu UGA te povećanom razgradnjom mRNA selenoproteina (14).
Najmanja regija koja je potrebna za funkciju SECIS su očuvani slijedovi 5' A/GUGA i 3' GA. Za tu je regiju naknadno dokazano da tvori nestandardne (engl. *non-Watson-Crick*) parove baza: purinske parove između GA na 5' bazi peteljke (u očuvanom slijedu A/GUGA) te GA u 3' bazi, kao i pirimidinske parove koji su granični za ta dva para. Ispostavilo se da slična nestandardna obilježja parova baza služe kao vezna mjesta za nekoliko sekvencijski i strukturno

be readily absorbed, have a concentration in tissues and biofluids at a physiologically relevant level, act in both aqueous and/or membrane domains (2). The most efficient enzymatic antioxidants involve superoxide dismutase, catalase and glutathione peroxidase. Non-enzymatic antioxidants involve thiol antioxidants (glutathione, thioredoxin and lipoic acid), vitamin C, vitamin E, carotenoids, natural flavonoids, melatonin and other compounds (selenium) (4).
In this review the focus is placed on a group of enzymes, glutathione peroxidases (GPXs), which are the major enzymes in the antioxidative defense mechanism depending on glutathione. The specific biochemical properties, synthesis and the role of each GPX isoenzyme will be described in biological processes. At least 7 types of GPXs have been identified (5,6), and their properties will be collected in a table divided into the following types: cytosolic- (cGPX or GPX1), gastrointestinal- (GI-GPX or GPX2), plasma- (pGPX or GPX3), phospholipid hydroperoxide- (PHGPX or GPX4) glutathione peroxidase, GPX5 and GPX6. Furthermore, non-enzymatic antioxidants, included in the activity of GPXs, will be reviewed.

Biosynthesis of glutathione peroxidases

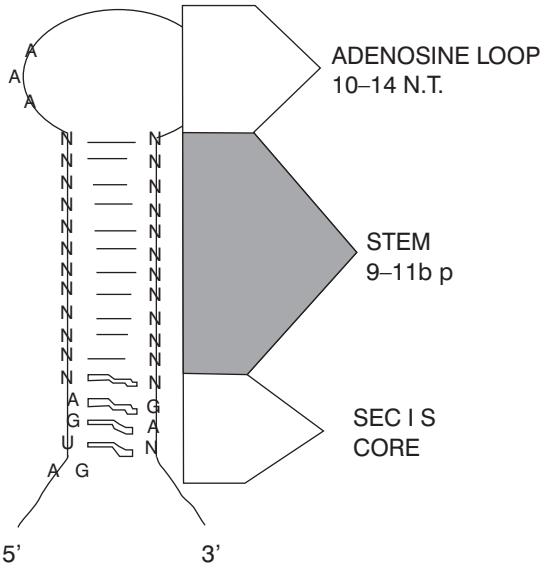
Biosynthesis of glutathione peroxidases is similar to biosynthesis of all selenoproteins which depends on the availability of selenium (Se). It was established in 1973 that Se is a structural component of the active center of the animal enzyme cellular glutathione peroxidase (GPX1) (6,7). Since then, 30 new selenoproteins have been identified, 15 of them were purified and their biological function was characterized (7-9).
Selenium is incorporated as selenocysteine (Sec) into the active site of a growing polypeptide chain encoded by UGA. This cotranslational incorporation of Sec into selenoproteins presents significant problems to the cell which must recognize the UGA as a Sec codon rather than a STOP translation signal (10,11,12).
The cloning of GPX1 has led to the identification of a specific eukaryotic Sec insertion sequence (SECIS) element as a stem-loop structure located in the 3` untranslated regions (UTR) of the glutathione peroxidase mRNAs. The SECIS element, which is in the active site, is the signal that recodes the in-frame UGA from a STOP to a Sec codon (13). Evidently, this complicated sequence provides ample opportunity for a posttranscriptional regulation of selenoprotein biosynthesis by selenium. However, selenium deficiency results in premature chain termination at the UGA codon as well as in increased degradation of the selenoprotein mRNAs (14).
The minimal region required for SECIS function are conserved 5' A/GUGA and 3' GA sequences. This region has been subsequently proven to form non-Watson-Crick ba-

specifičnih RNA-veznih proteina. U SECIS-elementima su očuvani adenozini sadržani u jednostavnoj otvorenoj omči (10,15) (Slika 1).

Umetak Sec u eukariota iziskuje zasebne čimbenike prevođenja koji uključuju Sec tRNA i čimbenik produljenja uz RNA-element u 3'-neprevedenom odsječku mRNA koji pak usmjerava ugradnju Sec kao odgovor na sve kodone UGA unutar okvira. U stanicama sisavaca taj je proces u velikoj mjeri reguliran te reagira na dostupnost selena na razini postojanosti i prevođenja RNA (11,13,14). Za spajanje Sec od najveće je važnosti SECIS-specifični vezni protein 2 (engl. *SECIS binding protein 2,* SBP2) koji također veže čimbenik produljenja EFsec sa specifičnošću za selenocisteil-tRNA (tRNA$^{(Ser)Sec}$). SBP2 se veže za očuvanu regiju s ne-Watson-Crickovim parovima baza u peteljci elementa SECIS i ostaje vezan tijekom višestrukih ciklusa prevođenja selenoproteina. tRNA$^{(Ser)Sec}$ se aminoacilira sa serinom koji se kasnije pretvara u Sec (13,16,17).

Kao što je prikazano na Slici 2., element SECIS privlači SBP2 u jezgri, a kompleks SECIS-SBP2 može privući kompleks EFsec-tRNA i uputiti ga prema ribosomu u kodirajućoj regiji. Činjenica da je element SECIS smješten u 3' UTR

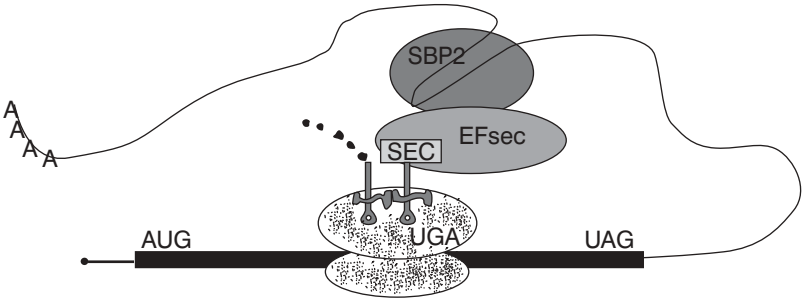
se pairs: purine pairs between the GA at the 5' base of the stem (in the conserved A/GUGA sequence) and the GA at the 3' base, and pyrimidine pairs flanking these two. Similar non-standard base-pairing features turned out to serve as binding sites for several sequence- and structure- specific RNA-binding proteins. In SECIS elements, the conserved adenosines are contained in a simple open loop (10,15) (Figure 1).



Slika 1. Ova slika prikazuje oblik elementa I SECIS (GPX1). Očuvani slijed i strukturna obilježja uključuju središnje SECIS-nukleotide (A/GUGA i GA), duljinu peteljke, te očuvane adenozine u završnoj omči (označeno kao *adenosine loop*). Tanke crte naznačuju Watson-Crickove parove baza dok podebljane crte označuju ne-Watson-Crickovo – nestandardno sparivanje. Oblik elementa SECIS II ima dvije peteljke, odvojen je regijom poqlyA i tipičan je za GPX3 i GPX4.

Figure 1. This figure shows the form of I SECIS element (GPX1). Conserved sequence and structural features include the SECIS core nucleotides (A/GUGA and GA), the stem length, and conserved adenosines in a terminal loop (marked as adenosine loop). The thin lines indicate Watson-Crick base pairs while the bold lines designate non-Watson-Crick pairing. The Form II SECIS element is the one with two stems, separated by a polyA region and is typical for GPX3 and GPX4.

The Sec insertion in eukaryotes requires dedicated translation factors including a Sec tRNA and elongation factor in addition to the RNA element in the 3'-untranslated portion of the mRNA that directs Sec incorporation in response to all in-frame UGA codons. In mammalian cells, this process is highly regulated and responsive to selenium availability, both at the levels of RNA stability and translation (11,13,14). Crucial for the Sec incorporation is a SECIS-specific binding protein, termed SECIS binding protein 2 (SBP2) which also binds to the elongation factor EFsec with specificity for selenocysteyl-tRNA (tRNA$^{(Ser)Sec}$). SBP2 binds to a conserved, non-Watson-Crick base-paired region in the stem of the SECIS element and remains bound through multiple cycles of selenoprotein



Slika 2. Spajanje Sec pomoću GPX vođeno s 3′ UTR. Otvoreni okvir čitanja eukariotske mRNA selenoproteina označen je crnom prugom, s ribosomom (označeno točkicama) koji dekodira kodon UGA Sec. UTR su naznačeni tankom crnom crtom. Kompleks SECIS-SBP2-EFsectRNA prikazan je sastavljen u 3-UTR uz savijanje unazad prema ribosomu.

Figure 2. Sec incorporation of GPX directed from 3′ UTR. The open reading frame of an eukaryotic selenoprotein mRNA is depicted by the black bar with a ribosome (dots) decoding the UGA Sec codon. The UTRs are indicated by the thin black line. The SECIS-SBP2-EFsectRNA complex is shown assembled in the 3-UTR and looping back to the ribosome.

Tablica 1. Geni koji su potrebni za sintezu selenoproteina u eukariota

Table 1. Genes required for selenoprotein synthesis in Eukaryotes

SelA	SEC
Efsec	Selenocysteyl-tRNA-specific elongation factor
SBP2	SECIS binding factor that interacts with EFsec
selC	tRNA (Ser)Sec
SPS1	Selenophosphate synthetase (non-selenoenzyme)
SPS2	Selenophosphate synthetase (selenoenzyme)

u eukariota, a ne u kodirajućoj regiji kao u prokariota, uklanja potrebu za disocijacijom i ponovnim spajanjem kompleksa SECIS-SBP2 sa svakim ugradbenim ciklusom. Takav sustav može omogućiti ponovno stvaranje kompleksa SECIS-SBP-EFsec-tRNA iz dva pojedinačna kompleksa RNA-protein nakon svakog popunjavanja ciklusa EFsec-tRNA za sljedeći približavajući ribosom. Ujedno je povoljan kod prevođenja proteina koji sadrži višestruke ostatke Sec poput selenoproteina P (5,11,13).

Specifična t-RNA$^{(ser)sec}$ se najprije opterećuje serinom, zatim pretvara u selenocisteil- tRNA$^{(Ser)Sec}$ sa selenofosfatom kao davateljem selena i vezanim sa svojom antikodonskom regijom na kodon UGA mRNA (14,15).

Zanimljivo je da izgleda da je postojanost mRNA povezana s djelotvornošću relevantnih elemenata SECIS u potiskivanju stop-kodona ili spajanju selenocisteina. To je zapažanje dovelo do fascinantne hipoteze da vezanje SelC za motiv SECIS ima dvojne učinke u eukariota uz posljedično ponovno kodiranje kodona UGA i stabilizaciju mRNA ovisnu o selenu (18).

translation. tRNA$^{(Ser)Sec}$ is aminoacylated with serine which is subsequently converted to Sec (13,16,17).

As shown in Figure 2, the SECIS element recruits the SBP2 in the nucleus and the SECIS-SBP2 complex could recruit the EFsec-tRNA complex and deliver it to a ribosome in the coding region. Because the SECIS element is located in the 3′ UTR in eukaryotes, not in the coding region as in prokaryotes, it obviates the need for dissociation and reassociation of the SECIS-SBP2 complex with each incorporation cycle. This scheme could allow a rapid reformation of SECIS-SBP-EFsec-tRNA complexes from two individual RNA-protein complexes after each EFsec-tRNA delivery cycle reloading for the next approaching ribosome. This would also be advantageous in the translation of a protein containing multiple Sec residues such as selenoprotein P (5,11,13).

A specific t-RNA$^{(ser)sec}$ is first loaded with serine, then transformed into selenocysteyl-tRNA$^{(ser)sec}$ with selenophosphate as the selenium donor and bound with its anticodon region to a UGA codon of the mRNA (14,15).

Struktura GPX

Premda su GPX1, GPX3 i GI-GPX2 homotetrameri, GPX4 je monomer s molekularnom veličinom manjom od podjedinica drugih glutation-peroksidaza. Zbog svoje male veličine i vodoodbojne površine GPX4 imaju sposobnost reagiranja sa složenim membranskim lipidima (19).

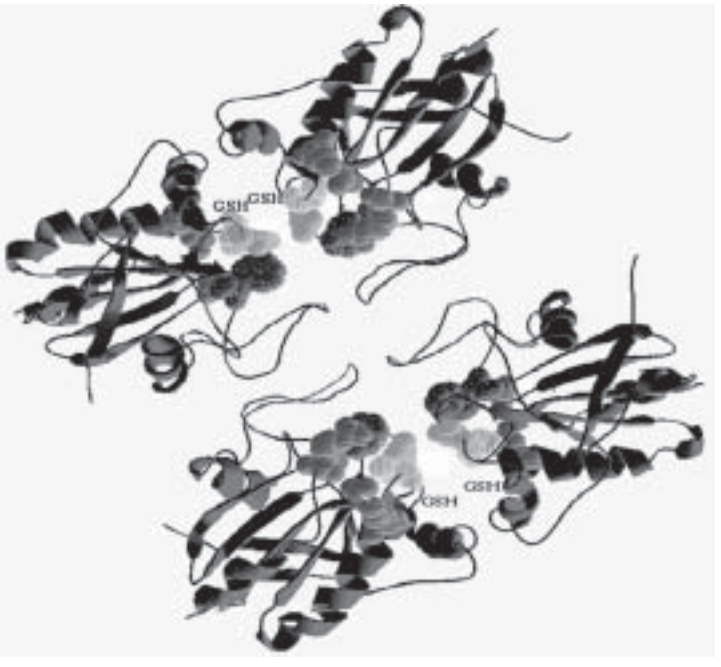
Struktura GPX u sisavaca također je analizirana računalom uz pomoć molekularnog modeliranja. Dobiveni modeli ukazuju da su osnovne faze katalize tri zasebne redoks-promjene selena na aktivnom mjestu koji se u osnovnom stanju pojavljuje na površini selenoperoksidaza kao središte karakteristične trijade koju izgrađuju selenocistein, glutamin i triptofan. U GPX četiri argininska ostatka i lizinski ostatak osiguravaju elektrostatičan ustroj koji u svakom reduktivnom koraku usmjerava glutation donorskog supstrata (engl. *donor substrate glutathione*, GSH) prema katalitičkom središtu tako da njegova sulfhidrilna skupina mora reagirati s dijelom molekule selena. Štoviše, mehanizmi vezanja kosupstrata su jedinstveni za klasičnu vrstu GPX1, no ne djeluju kod GPX3 i GPX4 (11) (Slika 3).

Analizom selenoproteoma karakterizirane su funkcije i slijed šest glutation-peroksidaza (GPX) u sisavaca: citosolne (cGPX, GPX1), prvog identificiranog selenoproteina kod sisavaca (6,15), fosfolipid-hidroperoksidne GPX (PHGPX, GPX4) koja je prvi put opisana 1982. godine i kasnije sekvenciranjem potvrđena kao selenoprotein (21,22), te druge sekvencirane plazmatske GPX (pGPX, GPX3) (23), gastrointestinalne GPX (GI-GPX, GPX2) (24) i GPX6 (25). Interesantno, stabilnost mRNA izgleda korelira s učinkovitošću pripadajućih SECIS elemenata u supresiji stop-kodona ili ugradnji selenocisteina. Ovo opažanje dovelo je do intrigantne hipoteze da SelC vezanje na SECIS motiv ima dvostruki učinak u eukariota, rezultirajući u rekodiranju UGA kodona i stabilizaciji mRNA u selen-ovisnom načinu (18).

The structure of GPXs

Although GPX1, GPX3 and GI-GPX2 are homotetramers, the GPX4 is a monomer with a molecular size smaller than the subunits of other glutathione peroxidases. Due to their small size and hydrophobic surface, GPX4s have the ability to react with complex lipids in membranes (19).

The structure of mammalian GPXs was also analyzed by computer-assisted molecular modelling. The obtained models show that essential steps of catalysis are three distinct redox changes of the active site selenium, which in the ground state presents itself at the surface of selenoperoxidases as the center of a characteristic triad built by selenocysteine, glutamine and tryptophan. In GPX, four arginine residues and a lysine residue provide an electrostatic architecture which in each reductive step directs the donor substrate glutathione (GSH) towards the catalytic center in such a way that its sulfhydryl group must react



Slika 3. Strukturni model GPX1 kao homotetramera (kružnice s okomitim linijama označavaju aktivna mjesta - selenocistein na 47-aminokiselini; najsvjetlije sive kružnice: Gln 82; bijele s točkicama: Trp160; kružnice s vodoravnim linijama: Arg 52 i Arg 179).

Figure 3. Structural model of GPX1 as a homotetramer (spheres with vertical lines indicate the active sites - selenocysteine at amino acid 47, the brightest grey spheres: Gln 82, white dotted spheres: Trp 160, spheres with horizontal lines: Arg 52 and Arg 179).

trointestinalne GPX (GI-GPX, GPX2) (24) te kod ljudi GPX6 koja je ograničena na olfaktorni sustav (19).

Glutation i selen su značajni u mehanizmima antioksidacijske zaštite povezanima s GPX

Glutation-peroksidaza je uključena u zaštitu od oksidacijskog stresa, pri čemu glutation koristi kao supstrat. Glutation također djeluje kao supstrat u drugim detoksificirajućim enzimima protiv oksidacijskog stresa kao što su glutation-transferaze. On sudjeluje u prijenosu aminokiselina kroz plazmatsku membranu i izravno čisti hidroksilni radikal i singletni kisik te time detoksificira vodikov peroksid i lipidne perokside katalitičkim djelovanjem GPX. Glutation može obnoviti najvažnije vitamine, tj. vitamine C i E, natrag u njihove aktivne oblike (2).

Zahvaljujući cisteinu koji sadrži tiolnu skupinu, glutation je važan unutarstanični neenzimski antioksidans. Glutation je obilato prisutan u citosolu (1-11 mM), jezgrama (3-15 mM) i mitohondrijima (5-11 mM) te je glavni topljivi antioksidans u staničnim odjeljcima (2,25).

Unutarstanični sadržaj glutationa ovisi o čimbenicima okoliša i funkcionira kao ravnoteža između njegova iskorištenja i sinteze. Izlaganje ROS (uključujući H_2O_2)/RNS, ili spojevima koji mogu stvarati ROS, može povećati sadržaj GSH povećanjem brzine sinteze GSH (2).

Značajno je da se GPX natječu s katalazom za H_2O_2 kao supstrat. Redoks-ciklus glutationa je glavni izvor zaštite od blagog oksidacijskog stresa, dok katalaza postaje sve važnija u zaštiti od teškog oksidacijskog stresa (26).

Međutim, u stanicama životinja, a posebice u ljudskim eritrocitima, GPX se već dugo smatra vodećim antioksidacijskim enzimom za detoksifikaciju H_2O_2 jer katalaza ima mnogo manji afinitet za H_2O_2 nego GPX (2,27).

U velikom je broju studija ustanovljena povezanost između pojavnosti karcinoma i različitih poremećaja funkcija enzima povezanih s GSH, dok se za glutation S-transferaze (GST) češće izvještava s obzirom na promjene glutation-peroksidaza (2,3,25).

Selen, kao dio aktivnog mjesta u GPX, jest osnovni mikronutrijent za kojeg je pokazano da smanjuje pojavnost karcinoma debelog crijeva te žarišta aberantnih kripti u životinjskim modelima (27-29). Ukazano je i na njegovu moguću uključenost u kemoprevenciju nekih karcinoma. U studijama na ljudima podatci pokazuju da su koncentracije selena obrnuto povezane sa smrtnošću i pojavnošću karcinoma (20,30,31).

Stoga se čini da selen funkcionira kao antimutageni agens koji sprječava zloćudnu preinaku normalnih stanica. Čini se da su ti njegovi zaštitni učinci ponajprije povezani s aktivnošću glutation-peroksidaza. Koncentracije GPX1 su osobito odgovorne za kolebanja koncentracija selena u usporedbi s ostalim selenoproteinima (6).

with the selenium moiety. Moreover, cosubstrate binding mechanisms are unique for the classical type of GPX1 but cannot operate in GPX3 and GPX4 (11) (Figure 3).

Analysis of selenoproteome characterized the functions and sequence of 6 glutathione peroxidases (GPXs) in mammals: cytosolic GPX (cGPX, GPX1), the first identified mammalian selenoprotein (6,15), phospholipid hydroperoxide GPX (PHGPX, GPX4) which was first described in 1982 and later verified as selenoprotein by sequencing (21,22), and other sequenced plasma GPX (pGPX, GPX3)(23), gastrointestinal GPX (GI-GPX, GPX2)(24) and, in humans, GPX6 which is restricted to the olfactory system (19).

Glutathione and selenium are important in GPX-related antioxidative defense mechanisms

Glutathione peroxidase is involved in protection against oxidative stress, and thus uses glutathione as a substrate. Glutathione also acts as a substrate in other detoxifying enzymes against oxidative stress, such as glutathione transferases. It participates in amino acid transport through the plasma membrane, scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of GPX. Glutathione is able to regenerate the most important antioxidants, vitamins C and E back to their active forms (2).

Due to cysteine that comprises a thiol group, glutathione is an important intracellular non-enzymatic antioxidant. Glutathione is highly abundant in the cytosol (1-11mM), nuclei (3-15mM), and mitochondria (5-11mM) and is a major soluble antioxidant in cell compartments (2,25).

The intracellular content of glutathione depends on environmental factors and functions as a balance between its utilization and synthesis. Exposure to ROS (involving H_2O_2)/RNS, or to compounds which can generate ROS, can increase the content of GSH by increasing the rate of GSH synthesis (2).

Significantly, GPX competes with catalase for H_2O_2 as a substrate. Glutathione redox cycle is a major source of protection against mild oxidative stress, whereas catalase becomes increasingly important in protection against severe oxidative stress (26).

However, in animal cells, and especially in human erythrocytes, the principal antioxidant enzyme for H_2O_2 detoxification has for a long time been considered to be GPX, as catalase has much lower affinity for H_2O_2 than GPX (2,27).

A large number of studies have established an association between cancer incidence and various disorders of GSH-related enzyme functions, while glutathione S-transferases (GSTs) have been more frequently reported with regard to alterations of glutathione peroxidases (2,3,25).

Selenium, as part of the active site in GPXs, is an essential micronutrient shown to reduce colon cancer incidence and preneoplastic aberrant crypt foci in animal models

Specifična biološka svojstva ljudskih glutation-peroksidaza

A. Funkcije glutation-peroksidaza

Unatoč njihovu posvudašnjem izražaju, koncentracije svake izoforme variraju ovisno o vrsti tkiva. Sve glutation-peroksidaze reduciraju vodikov peroksid i alkilne hidroperokside na račun glutationa. Njihove se specifičnosti za hidroperoksidni supstrat, međutim, izrazito razlikuju. Citosolne i mitohondrijske glutation-peroksidaze (cGPX ili GPX1) reduciraju samo topljive hidroperokside kao što je H_2O_2 te neke organske hidroperokside kao što su hidroperoksi-masne kiseline, izopropil-benzen-hidroperoksid ili t-butil-hidroperoksid. GPX1 i fosfolipid-hidroperoksidna glutation-peroksidaza GPX4 (ili PHGPX) nalaze se u većini tkiva. GPX4 je smješten i u citosolu i u membranskoj frakciji. Nadalje, GPX4 može izravno reducirati složenije lipide poput fosfatidilkolin-hidroperoksida, hidroperoksida masnih kiselina i hidroperoksida kolesterola koji se stvaraju u peroksidiranim membranama i oksidiranim lipoproteinima (32-35). GPX3 je usmjeren na izvanstanične odjeljke i luče ga različita tkiva u dodiru s tjelesnim tekućinama. On reducira fosfolipidne hidroperokside i doprinosi izvanstaničnom antioksidacijskom stanju kod ljudi. Međutim, važnost njegove funkcije još uvijek je upitna zbog niske koncentracije glutationa u plazmi (33).
GPX1 sprječava citotoksično, peroksidima izazvano oksidacijsko oštećenje, peroksidaciju lipida i razgradnju proteina. GPX4 je potreban za embriogenezu i mušku plodnost. Točna funkcija GPX3 još uvijek je nepoznata, dok bi GPX2 mogao biti protuupalni i antikancerogeni enzim (36).

B. Regulacija sinteze

Za egzogenu je opskrbu selenom utvrđeno da kontrolira enzimsku aktivnost ljudskog GPX1. U okolini s pomanjkanjem selena u stanicama postoji oko 5% normalne ljudske aktivnosti GPX1. Međutim, na koncentraciju GPX1 mRNA ne utječe koncentracija selena, što pak ukazuje da je ljudski GPX1 gen posttranskripcijski reguliran selenom (37,38).
Nedavno je utvrđeno da GPX1 inducira etopozid, koji je inhibitor topoizomeraze II, pobuđivač apoptoze i aktivator p53. Analizama vezanja DNA dokazano je da p53 povoljno regulira uzlazni promotorski element gena *GPX1*. Ta transaktivacija *GPX1* pomoću p53 povezuje signalni put p53 s putem antioksidacije (38). Štoviše, analiza apoptoze potaknute s p53 u ljudskom karcinomu debelog crijeva pokazala je da je povišen izražaj p53 povezan s povišenim GPX1 (38,39).
Osim toga, hiperhomocisteinemija je jedan od rizičnih čimbenika ateroskleroznog oboljenja krvnih žila. Za homocistein je navedeno da inhibira izražaj GPX1 i dovodi do povećanja ROS kojima su inaktivirani dušični oksid i pospješena endotelna disfunkcija. Ta se endotelna disfunkcija (27, 28, 29). It has also been implicated in possible chemoprevention of some cancers. In human studies, data have indicated that selenium levels are inversely associated with cancer mortality and incidence (20,30,31).
Thus, it appears to function as an antimutagenic agent, preventing the malignant transformation of normal cells. These protective effects of Se seem to be primarily associated with the activity of glutathione peroxidases. GPX1 levels are particularly responsive to fluctuations in selenium levels compared with other selenoproteins (6).

Specific biological properties of human glutathione peroxidases

A. Functions of glutathione peroxidases

Although their expression is ubiquitous, the levels of each isoform vary depending on tissue type. All glutathione peroxidases reduce hydrogen peroxide and alkyl hydroperoxides at the expense of glutathione. Their specificities for hydroperoxide substrate, however, differ markedly. Cytosolic and mitochondrial glutathione peroxidases (cGPXs or GPX1) reduce only soluble hydroperoxides, such as H_2O_2, and some organic hydroperoxides, such as hydroperoxy fatty acids, cumene hydroperoxide or t-butyl hydroperoxide. GPX1 and the phospholipid hydroperoxide glutathione peroxidase GPX4 (or PHGPX) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. Furthermore, it can also directly reduce more complex lipids such as phosphatidylcholine hydroperoxides, fatty acid hydroperoxides and cholesterol hydroperoxides which are produced in peroxidized membranes and oxidized lipoproteins (32-35). GPX3 is directed to extracellular compartments and is excreted from various tissues in contact with body fluids. It reduces phospholipid hydroperoxides and contributes to extracellular antioxidant status in humans. However, the importance of its function is still questionable because of the low plasma concentration of glutathione (33).
GPX1 prevents cytotoxic peroxide-induced oxidative damage, lipid peroxidation and protein degradation. GPX4 is required for embryogenesis and male fertility. The exact function of GPX3 is still unknown and GPX2 might be an anti-inflammatory and anti-carcinogenic enzyme (36).

B. Regulation of synthesis

Exogenous selenium supply has been found to control the enzymatic activity of human GPX1. In a selenium-deficient environment, cells have about 5% of normal human GPX1 activity. However, GPX1 mRNA level is not affected by selenium level, which suggests that human *GPX1* gene is regulated post-transcriptionally by selenium (37,38).
Recently, it has been found that GPX1 is induced by etoposide, a topoisomerase II inhibitor, an apoptosis inducer and a p53 activator. DNA-binding assays have proved that

kcija potaknuta homocisteinom riješila uvećanim izražajem GPX1 (40,41).

C. Stanično signaliziranje

GPX1 neutralizira događaje modulirane hidroperoksidom, kao npr. signaliziranje citokina i apoptozu potaknutu s CD95, radi uklanjanja neoplastičnih stanica (42,43). GPX1 ima također važnu ulogu u infekciji virusom ljudske imunodeficijencije (HIV) (44).

Glutation-peroksidaze moduliraju aktivaciju NF-κB, što je potvrđeno sljedećim razmatranjima: inhibitori ciklooksigenaza i lipooksigenaza inhibiraju aktivaciju NF-κB, aktivnost ciklooksigenaza ovisi o tonu hidroperoksida kojeg reguliraju glutation-peroksidaze, aktivacija NF-κB je inhibirana u stanicama s nadopunom selena, a olakšana kod pomanjkanja selena. Štoviše, pokusima je ukazano da pretjerani izražaj GPX1 u ljudskim kancerogenim stanicama T47D inhibira aktivaciju NF-κB potaknutu s TNF i modulira obrazac fosforilacije hsp27 nakon tretmana s TNF. Za GPX4 je, međutim, dokazano da je privlačniji kandidat za suzbijanje lipooksigenaza i utjecaj na signaliziranje citokina (45).

Apoptoza ili programirana smrt stanica ima važnu ulogu tijekom razvoja zametka, u pregradnji tkiva, uravnotežnju karcinogeneze i u sustavu obrane domaćina. Moguće ga je pobuditi u T-stanicama pomoću ROS, antigena Fas ili pomoću TNF. Većina istraživanja uloge GPX1 u apoptozi provedena je na stanicama dobivenima iz limfocita. Pojačana aktivnost GPX1 je inhibirala apoptozu potaknutu hidroperoksidima. Ta je činjenica potvrđena nadopunom goveđih bubrežnih epitelnih stanica sa selenom ili pretjeranim izražajem konstrukta GPX1 u mijeloičnoj staničnoj liniji (33,46,47).

Konačno, uloga GPX u infekciji HIV-om je podrobno istraživana. Umnožavanje HIV-a ovisi o aktivaciji NF-κB. Niske koncentracije GSH i glutation-peroksidaza u stanicama CD4+ povisuju koncentraciju hidroperoksida i time dovode to poticanja apoptoze. Stanice zaražene HIV-om umiru tijekom procesa apoptoze. Apoptozu djelotvorno inhibira antiapoptotički produkt gena Bcl-2 kojemu je dokazana antioksidacijska funkcija. Slični su rezultati dobiveni sa stanicama koje prejako izražavaju GPX1. Stoga GPX1 i Bcl-2 pokazuju slične učinke na antioksidacijski događaj u signalnoj kaskadi i dovode do inhibicije apoptoze, iako različitim mehanizmima. GPX1 izravno reducira hidroperokside, dok Bcl-2 sprječava njihov nastanak. Na temelju tih studija te studija koje uključuju depleciju GSH zaključeno je da bi se smanjenjem koncentracija GSH i aktivnosti GPX prije infekcije moglo smanjiti širenje virusa zahvaljujući apoptozi uzrokovanoj oksidacijskom mikrookolinom (48,49).

D. Patofiziološke funkcije enzima

U slučaju karcinoma glave i vrata za gubitak GPX1 alela je dokazano da se događa u histopatološko normalnom

p53 positively regulates an upstream promoter element of the *GPX1* gene. This transactivation of *GPX1* by p53 links the p53 signalling pathway to the antioxidant pathway (38). Moreover, analysis of the p53-induced apoptosis in a human colon cancer cell line showed that elevated p53 expression was associated with elevated GPX1 (38,39).

In addition, hyperhomocysteinemia is one of the risk factors for atherosclerotic vascular disease. Homocysteine has been reported to inhibit the expression of GPX1 and lead to an increase in reactive oxygen species that inactivated nitric oxide and promoted endothelial dysfunction. The overexpression of GPX1 rescued this homocysteine-induced endothelial dysfunction (40,41).

C. Cellular signalling

GPX1 counteracts hydroperoxide-modulated events, such as cytokine signalling and CD95-triggered apoptosis, to eliminate neoplastic cells (42,43), and GPX1 also has an important role in human immunodeficiency virus (HIV)-infection (44).

Glutathione peroxidases modulate NF-κB activation, which has been confirmed by the following considerations: inhibitors of cyclo-oxygenases and lipooxygenases inhibited activation of NF-κB, the activity of cyclo-oxygenases depends on the hydroperoxide tone which is regulated by glutathione peroxidases, NF-κB activation was inhibited in selenium-supplemented cells and facilitated in selenium deficiency. Moreover, experiments have implicated that GPX1 overexpression in human T47D carcinoma cells inhibited TNF-induced activation of NF-κB and modulated the phosphorylation pattern of hsp27 upon TNF treatment. However, GPX4 proved to be a more attractive candidate for silencing lipooxygenases and influencing cytokine signalling (45).

Apoptosis or programmed cell death plays an important role during embryonic development, in tissue remodelling, balancing carcinogenesis and host defense system. It can be induced in T cells by ROS, Fas antigen or TNF. Most of the studies of the GPX1 role in apoptosis were done in cells derived from lymphocytes. Enhanced GPX1 activity inhibited apoptosis induced by hydroperoxides. This fact was confirmed by supplementation of bovine renal epithelial cells with selenium or by overexpression of GPX1 construct in a myeloic cell line (33,46,47).

Finally, the role of GPX in HIV infection has been studied in detail. The replication of the HIV depends on the activation of NF-κB. Low levels of GSH and glutathione peroxidases in CD4+ cells enhance the level of hydroperoxides, leading to stimulation of apoptosis. The HIV-infected cells die in an apoptotic process. The apoptosis is efficiently inhibited by the anti-apoptotic Bcl-2 gene product, which has been shown to exert an antioxidant function. Similar results have been obtained with the cells overexpressing GPX1. Thus, GPX1 and Bcl-2 display analogous effects on

tkivu u blizini tumora, što ukazuje da bi gubitak na tom mjestu mogao biti rani događaj u razvoju karcinoma. Genetički modificirani miševi s GPX1 razvijeni su radi istraživanja posljedične fiziološke funkcije tog enzima. Ti su miševi rasli i razvijali se normalno, bez histopatoloških očitovanja sve do dobi od 15 mjeseci, što je ukazalo na ograničenu ulogu GPX1 tijekom normalnog razvoja pod fiziološkim uvjetima (50). Međutim, nakon stresa uzrokovanog parakvatom, GPX1(-,-) miševi su umrli brže nego oni iz kontrolne skupine. Neuroni u genetički manipuliranih GPX1 miševa također su bili osjetljiviji na H_2O_2. U tih su miševa i očne leće bile manje sposobne za oporavak nego u kontrolnih miševa nakon izlaganja fotokemijskom stresu. GPX1 (-,-) miševi su izražavali nepromijenjene koncentracije GPX4, GPX3 i GPX2, što bi posebice moglo zamijeniti pomankanje GPX1 (50).

Genetičke promjene u ljudi dovode do smanjene aktivnosti GPX, redukcije GSSG ili opskrbe NADPH-om, što ostaje asimptomatično (33).

Chu i suradnici su predložili moguću zaštitnu ulogu GPX2 protiv karcinoma debelog crijeva. Takva je uloga dokazana temeljem kromosomskog mapiranja mišjeg gena GPX2 blizu lokusa *Ccs1* na kromosomu 12 koji sadrži gen osjetljiv na karcinom debelog crijeva. Zapaženo je da su koncentracije GPX2 mRNA više nego u miševa osjetljivih na ICR/HA (31).

GPX3 je najprije otkriven u plazmi no njegova mRNA je pretežito nađena u epitelnim stanicama proksimalnih tubula bubrega. Bolesnici s bubrežnim bolestima imali su vrlo malu aktivnost GPX3, uključujući i bolesnike podvrgnute dijalizi. Takvo smanjenje GPX3 u plazmi nije bilo povezano s pomanjkanjem selena kod bolesnika. Ta činjenica predstavlja antioksidacijsku zaštitnu ulogu tog enzima u proksimalnim tubulima bubrega (51,52).

Hibridizacijom *in situ* otkriveno je da je GPX4 jako izražen u kasnim spermatogenim stanicama. Oksidacijska inaktivacija GPX4 je očevidno ključan korak u dozrijevanju sperme. Za jezgreni se oblik GPX4 vjeruje da doprinosi kondenzaciji kromatina (53). Za GPX4 je također dokazano da funkcionira kao strukturni protein u glavama spermija gdje čini 50% proteina (54).

Neka obilježja različitih GPX zajedno su prikazana u Tablici 2.

an antioxidative event in the signalling cascade leading to inhibition of apoptosis, although by different mechanisms. GPX1 directly reduces hydroperoxides, whereas Bcl-2 prevents their formation. Based on these studies and studies involving GSH depletion, it was concluded that a reduction in GSH levels and GPX activity before infection could decrease spread of the virus due to apoptosis caused by oxidative microenvironment (48,49).

D. Pathophysiologic functions of enzymes

In case of head and neck cancers, GPX1 allelic loss has been shown to occur in histopathologically normal tissue adjacent to tumors, indicating that loss at this locus may be an early event in cancer evolution. GPX1 knockout mice have been created to study the consequent physiologic function of this enzyme. These mice grew and developed normally and did not show any histopathologies up to 15 months of age, indicating a limited role of GPX1 during normal development and under physiologic conditions (50).

However, when stressed with paraquat, GPX1(-,-) mice died faster than controls. Neurons from GPX1 knockout mice were also more sensitive to H_2O_2. Moreover, eye lenses from knockout mice were less able to recover than those of control mice when exposed to photochemical stress. GPX1 (-,-) mice expressed unchanged levels of GPX4, GPX3 and GPX2, which may particularly substitute for the GPX1 deficiency (50).

Human genetic alterations lead to asymptomatic state of decreased GPX activity, GSSG reduction or NADPH supply (33).

Chu et al. suggested a possible protective role of GPX2 against colon cancer. Such role has been shown from chromosomal mapping of the mouse *GPX2* gene near the *Ccs1* locus on chromosome 12, which contains a colon cancer susceptible gene. GPX2 mRNA levels were observed to be higher than in sensitive ICR/HA mice (31).

GPX3 has been first detected in plasma, yet its mRNA was predominantly found in epithelial cells of renal proximal tubules. Patients with renal diseases had very low GPX3 activity, including those undergoing renal dialysis. This decrease in GPX3 in plasma was not associated with selenium deficiency in patients. This fact represents the antioxidative protective role of this enzyme in the proximal tubules of the kidney (51,52).

In situ hybridization revealed that GPX4 is abundantly expressed in late spermatogenic cells. Oxidative GPX4 inactivation is obviously a crucial step in sperm maturation. The nuclear form of GPX4 is believed to contribute to chromatin condensation (53). GPX4 was shown to function as a structural protein in sperm heads, where it constitutes 50% of the protein (54). Some characteristic of different GPXs are assembled below in table 2.

Type of the enzyme	Glutathione peroxidase 1 or cytosolic GPX (cGPX,GPX1)	Glutathione peroxidase 2 or gastrointestinal GPX (GI-GPX, GPX2)	Glutathione peroxidase 3 or plasma/extracellular GPX (pGPX, GPX-P, GPX3)	Glutathione peroxidase 4 or phospholipid hydroperoxide GPX (PHGPX, GPX4)	Glutathione peroxidase 5 or epididymal androge related protein or secretory GPX (GPX5)	Glutathione peroxidase 6orolfactory GPX (GPX6)
Gene structure/ chromosomal location	2 exons / 3p21.3 (Pseudogene: 3q11-q12)	2 exons / 14q24.1	5 exons / 5q32-q33.1	7 exons/19p13.3	5 exons/6p22.1	5 exons/ 6p22.1
Protein structure/ characteristics	Homotetramer; contains a single selenocysteine residue in each of four identical subunits (201 amino acids, 22kDa); protein sequence is highly conserved except for the last 5 amino acids, which is quite variable; a catalytically active selenocysteine residue is at amino acid 47 of the each subunit; Gln 82, Trp 160 are in a hydrogen-bond to the selenocysteine selenolate; Arg 52 and Arg 179 are believed to form salt-bridges to the carboxylate groups of glutathione (20).	Homotetramer (190 amino acids); selenocysteine at active site 40 of the protein sequence.	A glycosylated homotetramer of 23-kDa subunits (226 amino acids) that is able to use thioredoxin and glutaredoxin instead of GSH as the reducing thiol substrate; the active site with selenocysteine is at 73 amino acid (57).	Monomer (197 amino acids); selenocysteine is at active site 73	221 amino acids	221 amino acids
Regulation of protein synthesis	Post-transcriptional by selenium (38,39); inducible by etoposide, a topoisomerase II inhibitor, an apoptosis inducer and a p53 activator (39,40); homocysteine inhibition of the expression of GPX1→	Upregulation in colon, lung and skin cancers; a target for Nrf2 transcription factor and p63 (36,55).	In part by selenium levels.			
Tissue distribution	Abundant in tissues in humans with a high rate of peroxide production (such as erythrocytes, kidney, liver or lung); extremely low or absent in liver, kidney, heart, lung brain, testes (33).	Reduces H_2O_2 in the epithelium of the whole gastrointestinal tract from oesophagus to the distal colon (2- to 3- fold higher levels of GPX2 mRNA in ileum and cecum, present in crypts and villi). Some: in liver (33,56).	The only extracellular isoform of GPXs; a secreted protein into blood plasma; also expressed in the kidney, lung, heart, placenta; it was also found in small amounts in liver, skeletal muscle, pancreas, brain, lung, heart, the ciliary epithelium of the eye (52,58-60).	In most tissues both in cytosol and associated with membranes; it is targeted to the cytosol, mitochondria, or the nucleus, the major selenoprotein in sperm (61).	In epididymis; secreted protein.	In olfactory epithelium and embryos; secreted protein.
The role of the enzyme	Detoxification of H_2O_2/wide range of organic hydroperoxides through the coupled oxidation of reduced glutathione; prevents cytotoxic peroxide-induced oxidative damage, lipid peroxidation and protein degradation; enhanced GPX activity inhibited apoptosis induced by hydroperoxides (35).	Protects GIT against toxicity of ingested lipid peroxides; represents the defense barrier against ingested lipid hydroperoxides; protective role against colon cancer (31,35).	As barrier for any hydroperoxide transfer; reduction in hydrogen peroxide and lipid peroxides of more complex lipids such as phosphatidylcholine, although with lower efficiency than GPX4 at the expense of glutathione; a major scavenger of reactive oxygen species (ROS) (15,33).	Its small size and hydrophobic surface have been implicated in the ability of this enzyme to reduce peroxidized phospholipids and cholesterol in membranes; protects against oxidative stress; in redox regulation, sexual maturation and differentiation (essential for fertilization, spermatogenesis); also reduces hydroperoxides in HDL and LDL (53).		

Tablica 2. Karakteristike različitih GPX

Table 2. Characteristics of different GPXs

Zaključci

Zaključno, identificirano je barem sedam vrsta glutation-peroksidaza. Uz njihove intrinzične funkcije čistača, točan smještaj različitih GPX u tkivima govori u prilog specifičnih uloga tih enzima. Svaki GPX također može funkcionirati kao senzor vodikovog peroksida koji regulira koncentraciju H_2O_2. Zanimljivo je da specifična kotranslacijska ugradnja Sec u GPX ima dvojni učinak kod eukariota, kao što su ponovno kodiranje kodona UGA i stabilizacija mRNA ovisna o selenu, što bi se u budućnosti moglo istražiti za svaki tip GPX.

Zbog antioksidacijske i antimutagene uloge GPX postoji znatno zanimanje za njihovom terapijskom primjenom kao antioksidansima. Tu je moguća uporaba prirodno dostupnih antioksidansa ili potpuno sintetskih molekula. Nadalje, prevelik izražaj GPX mogao bi zaštititi različite stanice od oksidacijskog stresa. Aktivnost tih enzima mogu pojačati adenovirusi (62), selenidi, diselenidi i ebselen kao oponašatelji GPX s malim molekulama (63-65). Od oblikovanja novih tvari koje oponašaju različite GPX i njihova prijenosa do specifičnih mjesta u antikancerogenoj terapiji ili sprječavanju karcinoma očekuje se da uskoro postanu trend u znanstvenom istraživanju.

Conclusions

In conclusion, at least 7 types of glutathione peroxidases have been identified. Besides their intrinsic scavenger functions, precise localization of various GPXs in tissues argues in favor of specific roles of these enzymes. Each GPX could also function as a hydrogen peroxide sensor to regulate the H_2O_2 concentration. Interestingly, the specific cotranslational incorporation of Sec into GPXs has a dual effect in eukaryotes, such as recoding the UGA codon and stabilization of the mRNA in a selenium-dependent manner, which could be studied in the future for each type of GPX.

Due to the antioxidative and antimutagenic role of GPXs, there is a considerable interest in their therapeutic use as antioxidants. This may involve the use of naturally occurring antioxidants or completely synthetic molecules. Furthermore, overexpression of GPXs might protect various cells from oxidative stress. The activity of these enzymes might be elevated by adenoviruses (62), selenides, diselenides and ebselen as small-molecule GPX mimetics (63-65). The modelling of novel substances mimicking different GPXs and their transport to specific sites in anticancer therapy or its prevention are expected to become a trend in near future.

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Literatura/References

1. *Ratnasinghe D, Tangrea JA, Andersen MR, Barrett MJ, Virtamo J, Taylor PR, et al. Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. Cancer Res 2000;60:6381-3.*
2. *Valko M., Rhodes CJ, Moncol J. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160(1): 1-40.*
3. *Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005;12:1161-208.*
4. *Mates JM, Perez-Gomez C, Nunez De Castro I. Antioxidant enzymes and human diseases. Clin Biochem 1999;32:595–603.*
5. *Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 2007;9:775-806.*
6. *Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973;179(73):588-90.*
7. *Zachara BA. Mammalian selenoproteins. J Trace Elem Electrolytes Health Dis 1992;6(3): 137-51.*
8. *Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutr 2001;4(2B):593-9.*
9. *Beckett GJ, Arthur JR. Selenium and endocrine systems. J Endocrinol 2005;184:455-65.*
10. *Low SC, Berry MJ. Knowing when not to stop: selenocysteine incorporation in eukaryotes. Trends Biochem Sci 1996;21(6):203-8.*
11. *Hatfield DL, Gladyshev VN. How selenium has altered our understanding of the genetic code. Mol Cell Biol 2002;22:3565-76.*

12. Mullenbach GT, Tabrizi A, Irvine BD, Bell GI, Hallewell RA. Sequence of a cDNA coding for human glutathione preoxidase confirms TGA encodes active site selenocysteine. Nucleic Acids Res 1987;15:5484.
13. Tujebajeva RM, Copeland PR, Xu XM, Carlson BA, HArney JW, Driscoll DM, et al. Decoding apparatus for eukaryotic selenocysteine insertion. EMBO Rep 2000;1(2):158-63.
14. Wingler K, Bocher M, Flohe L, Kollmus H, Brigelius-Flohe R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. Eur J Biochem 1999;259(1-2):149-57.
15. Flohe L, Gunzler WA, Schock HH. Glutathione peroxidase: a selenoenzyme. FEBS Lett 1973;32(1):132-34.
16. Lee BJ, Worland P, Davis JN, Stadtman TC, Hatfield DL. Identification of a selenocysteyl-tRNA (Ser) in mammalian cells that recognizes the nonsense codon, UGA. J Biol Chem 1989;264:9724-7.
17. Jameson RR, Diamond AM. A regulatory role for Sec tRNA (Ser)Sec in selenoprotein synthesis. RNA 2004;10:1142-52.
18. Rybka K. Selenoproteins-atypical function of the UGA codon. Postepy Hig Med Dosw 1999;53(4):601-16.
19. Brigelius-Flohe R. Glutathione peroxidases and redox-regulated transcription factors. Biol Chem 2006;387(10-11):1329-35.
20. Aumann KD, Bedorf N, Brigelius-Flohe R, Schomburg D, Flohe L. Glutathione peroxidase revisited-simulation of the catalytic cycle by computer assisted molecular modelling. Biomed Environ Sci 1997;10(2-3):136-55.
21. Ursini F, Maiorino M, Valente M, Ferri L, Gregolin C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim Biophys Acta 1982;710(2):197-211.
22. Brigelius-Flohe R, Aumann KD, Blocker H, Gross G, Kiess M, Kloppel KD, et al. Phospholipid-hydroperoxide glutathione peroxidase. Genomic DNA, cDNA, and deduced amino acid sequence. J Biol Chem 1994;269(10):7342-8.
23. Takahashi K, Avissar N, Whitin J, Cohen H. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys 1987;256(2):677-86.
24. Chu FF, Doroshow JH, Esworthy RS. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSH-Px-GI. J Biol Chem 1993;268(4):2571-6.
25. Flohe, Brigelius-Flohe. Selenium: its molecular biology and role in human health, Selenoproteins of the glutathione system. Norwel 2001;157-78.
26. Yan H, Harding JJ. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J 1997;328:599-605.
27. Davis CD, Uthus EO. Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one carbon metabolism rats. J Nutr 2003;133(9):2907-14.
28. McIntosh GH, Royle PJ, Scherer BL. Selenised dairy protein and colon cancer inhibition in AOM induced rats. Asia Pac J Clin Nutr 2004;13: S93.
29. Davis CD, Zeng H, Finley JW. Selenium-enriched broccoli decreases intestinal tumourigenesis in multiple intestinal neoplasia mice. J Nutr 2002;132(2):307-9.
30. Ghadirian P, Maisonneuve P, Perret C, Kennedy G, Boyle P, Krewski D, et al. A case-control study of toenail selenium and cancer of the breast, colon, and prostate. Cancer Detect Prev 2000;24(4):305-13.
31. Chu FF, Esworthy RS, Chu PG, Longmate JA, Huycke MM, Wilczynski S, et al. Bacteria-induced intestinal cancer in mice with disrupted GPX1 and GPX2 genes. Cancer Res 2004;64(3):962-8.
32. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 2000;153(1-3):83-104.
33. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 1999;27(9-10):951-65.
34. Imai H, Narashima K, Arai M, Sakamoto H, Chiba N, Nakagawa Y. Suppression of leukotriene formation in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. J Biol Chem 1998;273(4):1990-7.
35. Flohe L. Glutathione peroxidase. Basic Life Sci 1988;49:663-8.
36. Brigelius-Flohe R. Glutathione peroxidases and redox-regulated transcription factors. Biol Chem 2006;387:1329-35.
37. Chada S, Whitney C, Newburger PE. Regulation of human glutathione peroxidase gene expression by selenium. Blood 1989;74(7):2535-41.
38. Tan M, Li S, Swaroop M. Transcriptional activation of the human glutathione peroxidase promoter by p53. J Biol Chem 1999;274(17):12061-6.
39. Gladyshev VN, Factor VM, Housseau F, Hatfiels DL. Contrasting patterns of regulation of the antioxidant selenoproteins thioredoxin reductase and glutathione peroxidase in cancer cells. Biochem Biophys Res Commun 1998;251(2):488-93.
40. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982;2(8291):175-9.
41. Upchurch GR Jr, Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF Jr, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem 1997;272(27):17012-7.
42. Yang P, Bamlet WR, Ebbert JO, Taylor WR, de Andrade M. Glutathione pathway genes and lung cancer risk in young and old populations. Carcinogenesis 2004;25(10):1935-44.
43. Gouaze V, Andrieu-Abadie N, Cuvillier O, Malagarie-Cazenave S, Frisach MF, Mirault ME, et al. Glutathione peroxidase-1 protects from CD95-induced apoptosis. J Biol Chem 2002;277(45):42867-74.
44. Gladyshev VN, Stadtman TC, Hatfield DL, Jeang KT. Levels of major selenoproteins in T cells decrease during HIV infection and low molecular mass selenium compounds increase. Proc Natl Acad Sci USA 1999;96:835-9.
45. Makropoulos V, Bruning T, Schulze-Osthoff K. Selenium-mediated inhibition of transcription factor NF-kappa B and HIV-1 LTR promoter activity. Arch Toxicol 1996;70(5):277-83.
46. Arthur JR. The glutathione peroxidases. Cell Mol Life Sci 2000;57:1825-35.
47. Saito Y, Nishio K, Ogawa Y, Kimata J, Kinumi T, Yoshida Y, et al. Turning point in apoptosis/necrosis induced by hydrogen peroxide. Free Radic Res 2006;40(6):619-20.
48. Sappey C, Legrand-Poels S, Best-Belpomme M, Favier A, Rentier B, Piette J. Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress. AIDS Res Hum Retroviruses 1994;10(11):1451-61.
49. Aillet F, Masutani H, Elbim C, Raoul H, Chene L, Nugeyre MT, et al. Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistent infection of CD4(+) T- or monocytic cell lines. J Virol 1998;72(12):9698-705.
50. Hu YJ, Dolan ME, Bae R, Yee H, Roy M, Glickman R, et al. Allelic loss at the GPX-1 locus in cancer of the head and neck. Biol Trace Elem Res 2004;101(2):97-106.
51. Whitin JC, Tham DM, Bhamre S, Ornt DB, Scandling JD, Tune BM, et al. Plasma glutathione peroxidase and its relationship to renal proximal tubule function. Mol Genet Metab 1998;65:238-45.
52. Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW, et al. Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. Am J Physiol Lung Cell 1996;270:L173-82.
53. Godeas C, Tramer F, Micali F, Roveri A, Maiorino M, Nisii C, et al. Phospholipid hydroperoxide glutathione peroxidase (PHGPX) in rat testis nuclei is bound to chromatin. Biochem Mol Med 1996;59(2):118-24.
54. Flohe L. Selenium in mammalian spermiogenesis. Biol Chem 2007;388:987-95.
55. Singh A, Rangasamy T, Thimmulappa RK, Lee H, Osburn WO, Brigelius-Flohe R, et al. Glutathione peroxidase 2, the major cigarette smoke – inducible isoform of GPX in lungs, is regulated by Nrf2. Am J Respir Cell Mol Biol 2006;35(6):639-50.

56. Reddy K, Tappel AL. Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of gastrointestinal tract and other tissues in the rat. J Nutr 1974;104:1069-78.
57. Takebe G, Yarimizu J, Saito Y, Hayashi T, Nakamura H, Yodoi J, et al. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P. J Biol Chem 2002;277:41254-8.
58. Avissar N, Ornt DB, Yagil Y, Horowitz S, Watkins RH, Kerl EA, et al. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. Am J Physiol 1994;266:C367-75.
59. Whitin JC, Bhamre S, Tham DM, Cohen HJ. Extracellular glutathione peroxidase is secreted basolaterally by human renal proximal tubule cells. Am J Physiol Renal Physiol 2002;283:F20-8.
60. Avissar N, Slemmon JR, Palmer IS, Cohen HJ. Partial sequence of human plasma glutathione peroxidase and immunologic identification of milk glutathione peroxidase as the plasma enzyme. J Nutr 1991;121:1243-9.
61. Pushpa V, Burdsal CA. Rat phospholipid hydroperoxide glutathione peroxidase: cDNA cloning and identification of multiple transcription and translation sites. J Biol Chem 1985;270:26993-9.
62. Robertson RP, Harmon JS. Pancreatic islet beta-cell and oxidative stress: The importance of glutathione peroxidase. FEBS Lett 2007;581(19):3743-8.
63. Back TG, Moussa Z. Diselenides and allyl selenides as glutathione peroxidase mimetics. Remarkable activity of cyclic seleninates produced in situ by the oxidation of allyl omega-hydroxyalkyl selenides. J Am Chem Soc 2003;125(44):13455-60.
64. Konorev EA, Kennedy MC, Kalyanaraman B. Cell-permeable superoxide dismutase and glutathione peroxidase mimetics afford superior protection against doxorubicin-induced cardiotoxicity: the role of reactive oxygen and nitrogen intermediates. Arch Biochem Biophys 1999;368(2):421-8.
65. Jauslin ML, Wirth T, Meier T, Schoumacher F. A cellular model for Friedreich Ataxia reveals small-molecule glutathione peroxidase mimetics as novel treatment strategy. Hum Mol Genet 2002;11(24):3055-63.