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High throughput drug screening identifies resveratrol as suppressor of hepatic SELENOP expression

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ABSTRACT

Introduction: Selenium (Se) is an essential trace element that exerts its effects mainly as the proteinogenic amino acid selenocysteine within a small set of selenoproteins. Among all family members, selenoprotein P (SELENOP) constitutes a particularly interesting protein as it serves as a biomarker and serum Se transporter from liver to privileged tissues. SELENOP expression is tightly regulated by dietary Se intake, inflammation, hypoxia and certain substances, but a systematic drug screening has hitherto not been performed.

Methods: A compound library of 1861 FDA approved clinically relevant drugs was systematically screened for interfering effects on SELENOP expression in HepG2 cells using a validated ELISA method. Dilution experiments were conducted to characterize dose-responses. A most potent SELENOP inhibitor was further characterized by RNA-seq analysis to assess effect-associated biochemical pathways.

Results: Applying a 2-fold change threshold, 236 modulators of SELENOP expression were identified. All initial hits were replicated as biological triplicates and analyzed for effects on cell viability. A set of 38 drugs suppressed SELENOP expression more than three-fold, among which were cancer drugs, immunosuppressants, anti-infectious drugs, nutritional supplements and others. Considering a 90% cell viability threshold, resveratrol, vidofludimus, and antimony potassium-tartrate were the most potent substances with suppressive effects on extracellular SELENOP concentrations. Resveratrol suppressed SELENOP levels dose-dependently in a concentration range from 0.8 μM to 50.0 μM, without affecting cell viability, along with strong effects on key genes controlling metabolic pathways and vesicle trafficking.

Conclusion: The results highlight an unexpected direct effect of the plant stilbenoid resveratrol, known for its antioxidative and health-promoting effects, on the central Se transport protein. The suppressive effects on SELENOP may increase liver Se levels and intracellular selenoprotein expression, thereby conferring additional protection to hepatocytes at the expense of systemic Se transport. Further physiological effects from this interaction require analyses in vivo and by clinical studies.

1. Introduction

The essential trace element selenium (Se) is required in sufficient amounts to support and maintain the biosynthesis of selenoproteins [1–3]. The trace element is integrated into proteins in the form of selenocysteine (Sec) or selenomethionine (SeMet), the latter event occurring randomly at AUG codons, while the former is a tightly regulated process [3–5]. The amino acid Sec confers unique catalytic properties to the family of Sec-containing selenoproteins that serve critical functions in human physiology, including controlling thyroid hormone metabolism, redox status, immune cell function and ensuring targeted distribution of Se within the organism to sites of highest demand and requirements [6–8]. To this end, hepatocytes synthesize and secrete the Se transporter selenoprotein P (SELENOP), which also serves as a suitable biomarker for assessing Se status [9–12].

The decoding efficiency of UGA as a Sec-insertion codon can be

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modulated by various endogenous and external factors such as gender, age, nutritional status, current disease states and certain medication [3,13–15]. Among the drugs known to affect selenoprotein biosynthesis are some antibiotics of the aminoglycoside family, immunosuppressants, metformin and statins [16–19]. Some of the serious side effects observed with these drugs can be explained in part by their adverse effects on Se homeostasis and selenoprotein expression, e.g. aminoglycoside-induced ototoxicity [20] or statin-related myopathy [21]. However, the data base for these potential interferences in human subjects is small.

Prescription rates and drug use increase with age [22]. Polypharmacy in the background of inadequate micronutrient supply and malnutrition is a relevant health problem [23,24], as the side effects on metabolic pathways may adversely affect energy metabolism, tissue maintenance and promote degenerative processes. The trace element Se and the enzymatically active selenoproteins have been directly implicated in age-related diseases, as the proteins affect important biochemical pathways controlling thyroid hormone metabolism, redox status, antioxidative defence and death from ferroptosis [3,25,26]. To obtain a first global view of possible disruption of regular selenoprotein biosynthesis by drugs, a compound library of nearly two thousand substances, composed of clinically used FDA-approved drugs, was tested with human hepatocellular carcinoma cells *in vitro* for effects on SELENOP expression, i.e. by analysing extracellular SELENOP concentrations that reflect cellular SELENOP translation and secretion. The results indicate critical interactions and highlight several particularly relevant compounds that may be important for better understanding the health-promoting or harmful side effects of pharmacological interventions, especially given the trend towards polypharmacy in the elderly.

2. Materials and methods

2.1. Cell culture

Human hepatocellular HepG2 cells were seeded with DMEM/F12 (Gibco, Thermo Fisher Scientific GmbH, Dreieich, Germany) +10% (v/v) FCS (Sigma-Aldrich, Steinheim, Germany) at 2×10^4 cells per well in 96-well plates, at 5×10^5 cells per well in 6-well plates, or at 1.5×10^6 cells per 25 cm^2 flask, and cultivated at 37 °C and 5% CO_2 under standard conditions. Medium was exchanged after 24 h, and cells were cultivated for 48 h in serum-free DMEM/F12 + 100 nM sodium selenite (Sigma-Aldrich) ± compound. The initial screen and follow-up experiments were performed using the FDA-approved Drug Library L1300 (Selleck Chemicals LLC, Houston, TX, USA), or Resveratrol (SRT501, Selleck Chemicals), Resveratrol 3-sulfate (sc-213070, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or Resveratrol 3-O-β-D-glucuronide (sc-213069, Santa Cruz). Final medium DMSO concentrations were set to 0.5% (v/v) (D8418, Sigma-Aldrich). After 48 h, 200 μL of medium was harvested and stored at −20 °C for further analysis. For isolation of total RNA cells were washed twice with 500 μL PBS. RNA was isolated using Aurum™ Total RNA Mini Kit (#732–6820, Bio-Rad Laboratories GmbH, Feldkirchen, Germany) and RNA Clean & Concentrator-5 (R1013, Zymo Research Corporation, Irvine, CA, USA) according to the manufactures' instructions. For the analysis of total protein concentration and intracellular SELENOP concentration, cells in 6-well plate were washed with 500 μL PBS, scratched in 200 μL homogenization buffer (250 mM sucrose, 20 mM HEPES, 1 mM EDTA, 0.1% (v/v) Triton X-100, pH 7.5) and stored at −20 °C for homogenization.

2.2. Assessment of glutathione peroxidase (GPx) activity

Cell pellets were homogenized on ice using an ultrasonic homogenizer (Labsonic®M, B. Braun Biotech International GmbH, Melsungen, Germany) applying 2 × 3 homogenization steps (0.8 cycles at 100% amplitude). The activity of glutathione peroxidase (GPx) was assessed by a coupled enzymatic test procedure monitoring nicotinamide adenine dinucleotide phosphate (NADPH) consumption at 340 nm, as described earlier [27]. Briefly, 5 μL cell lysate samples were incubated with enzyme buffer containing 3.4 mM reduced glutathione (GSH), 0.27 mg/mL NADPH, 1.0 mM NaN_3, and 0.3 U/mL glutathione reductase. The enzymatic reaction was started by hydrogen peroxide (H_2O_2), and consumption of NADPH was monitored at 340 nm. Coefficients of variation (CV) between measurements were below 7.5%, as determined with a standard preparation.

2.3. Quantification of SELENOP and ceruloplasmin

Extracellular and intracellular SELENOP concentrations were measured with a sandwich ELISA using 50 μL of cell culture medium or homogenate. Briefly, monoclonal antibodies to human SELENOP (selenOmed GmbH, Berlin, Germany) were purchased, and 96-well plates were prepared and used for SELENOP assessment as described earlier [28]. A serum pool of self-reported healthy individuals (n = 25) with a known SELENOP concentration was purchased from a commercial supplier (inVent Diagnostica GmbH, Hennigsdorf, Germany) and used as calibrator ranging from 5.6 μg/L to 720 μg/L of SELENOP. A working range was determined according to the coefficient of variation (CV < 15%), ranging from 9.8 μg/L to 583.8 μg/L. SELENOP concentrations were calculated from the dose-response curve using a nonlinear four-parameter logistic (4-PL) regression model in GraphPad Prism 9.3.1. software. Assay accuracy was confirmed by three commercially available SELENOP control samples of low (Con L), medium (Con M) and high (Con H) SELENOP contents with the following concentrations; Con L = 14.6 ± 2.2; Con M 58.5 ± 8.8 and Con H 234.2 ± 35.1 ng/mL (selenOtest, LOT: STE.21002, selenOmed). SELENOP calibrators were prepared according to the manufacturer's instructions and 50 μL were applied in duplicate in every 96-well plate ELISA analysis (n = 2, in 3 runs). During the screening assay precision was calculated with a diluted serum sample, yielding a mean SELENOP concentration of 86.6 ± 11.1 μg/L and a CV = 12.8% by n = 132 measurements in 33 runs. Ceruloplasmin (CP) concentrations were measured with a modified sandwich ELISA using 50 μL of cell culture medium as described in detail earlier [28].

2.4. Western blot analysis

SDS-PAGE was performed using 10% or 15% polyacrylamide gels in combination with Lämmli buffer (25 mM TRIS, 192 mM Glycine, 0.1% (w/v) SDS, pH 8.3). A total of 15 μg protein of homogenized cell culture samples or 20 μL of cell culture medium were diluted 1:4 in sample buffer (250 mM TRIS, 4% (w/v) SDS, 40% (v/v) glycerol, 20% (v/v) β-mercaptoethanol, 0.02% (v/v) bromophenol blue) and heated at 95 °C for 5 min. Protein concentrations were determined by Bradford analysis (Bio-Rad Laboratories, Munich, Germany). Protein samples were blotted onto a 0.2 μm PVDF membrane (Thermo Fisher) in Lämmli transfer buffer containing 20% (v/v) methanol. Ponceau S (Sigma) was used for the visualization of the transferred proteins, until albumin as main secretory product of HepG2 cells in serum-free medium became clearly visible. Primary monoclonal antibodies recognizing SELENOP were detected using anti-mouse IgG-HRP (NXA931, GE Healthcare GmbH, Solingen, Germany). Primary antibodies to Retinol-Binding Protein-4 (RBP4; Dako A0040, Dako Denmark, Glostrup) and to Transthyretin (TTR monomer; Dako A0002, Dako Denmark, Glostrup), respectively, were detected using anti-rabbit IgG-HRP (P0448, Dako Denmark, Glostrup) in combination with ECL Western Blotting Detection Reagents and Hyperfilm™ (GE Healthcare).

2.5. RNA sequencing

Triplicates of isolated RNA samples were diluted to a final concentration of 15 ng/mL. RNA-library was prepared with NEB-Next Ultra II RNA Kit including a Poly-(A)-Selection step (New England Biolabs Inc.,

USA). The library quality control was done using TapeStation, resulting in RNA integrity numbers >8.0 for all samples. To this end, RNA samples were applied to v2.5 flow cell, and single end 75 cycles sequencing were performed using Next Seq 500 Sequencing System (Illumina Inc., San Diego, CA, USA). Raw data were evaluated, annotated and provided for further analysis by the local Core Unit Genomics (Berlin Institute of Health, Charité-Universitätsmedizin, Berlin, Germany). FDR was set to 0.05. The *DEseq2* package in R was used to calculate differential gene expression [29].

2.6. Statistical analysis

All continuous data was presented as means ± standard deviations (SD). Fold change in expression of SELENOP was calculated by dividing expression under drug application by expression under the DMSO control. First drug screening was presented as jitter plot, second verification screening was presented as mean ± (SD) waterfall plot. Wilcoxon rank-sum test was used to compare two groups of continuous data.

Adjusted p-values below 0.05 and Log2 fold change expression of >0.58 or < −0.58 were used as cut-offs to determine differentially expressed genes (DEGs) generated by *DEseq2* [29]. Volcano and MA

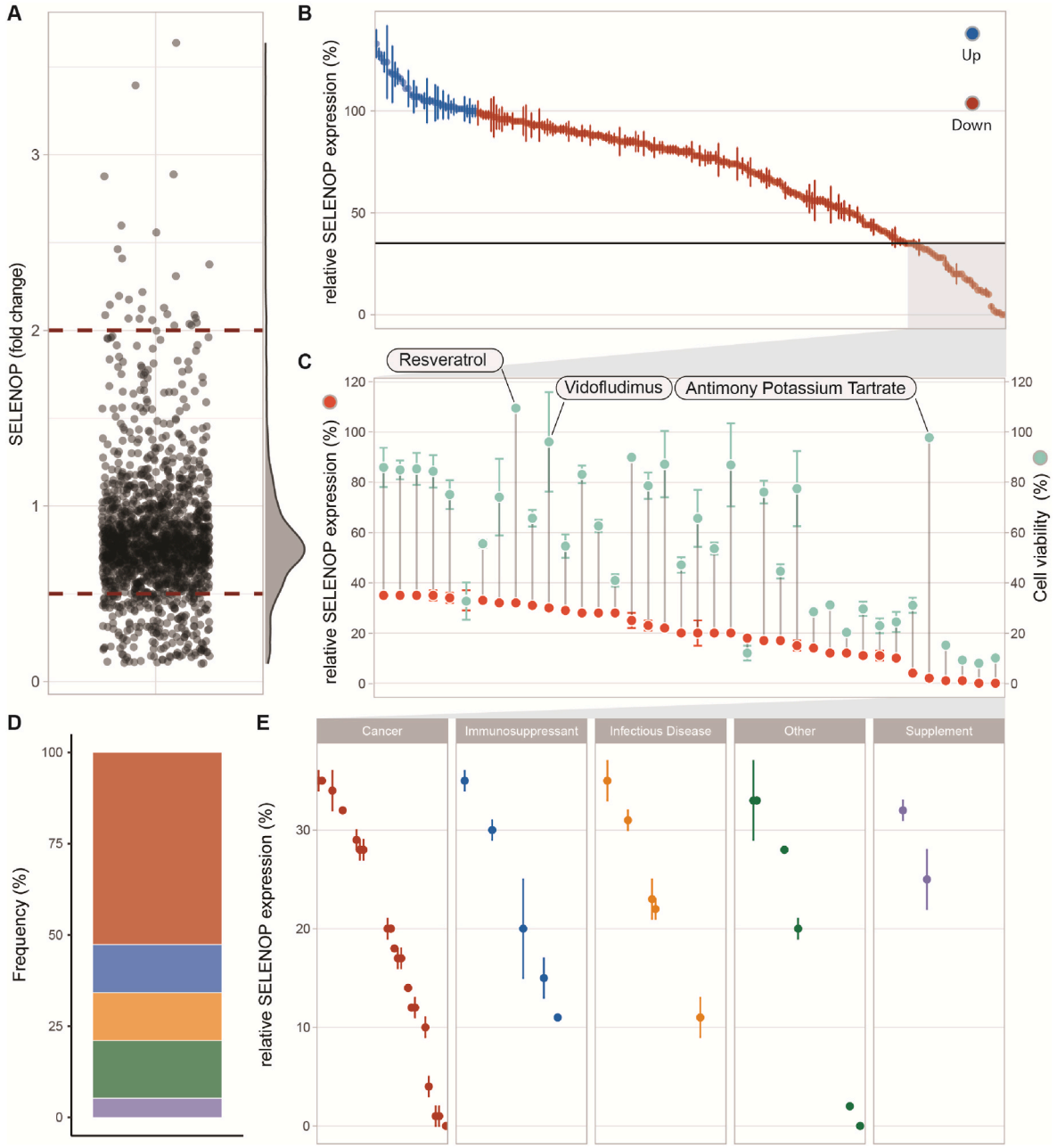


Fig. 1. Identification of drugs affecting hepatic SELENOP expression. (**A**) From a total of n = 1861 compounds, a set of 236 were identified with modulating effects on hepatic SELENOP expression (experimental setup; n = 1). Red lines at y = 0.5 and y = 2.0 indicate the cut-off values. Compounds yielding a relative SELENOP expression <0.1 were excluded (n = 146). (**B**) Validation of the identified 236 compounds revealed (**C**) resveratrol, vidofludimus and antimony potassium tartrate as most relevant hits complying with stringent screening criteria; relative SELENOP expression <35% (red dots) and mean cell viability >90% (green dots) in comparison to 0.5%(v/v) DMSO-treated cells (experimental setup; n = 3). (**D**) Overview of the fields of application of the 38 identified suppressors. (**E**) Mean SELENOP expression of the 38 identified suppressors according to field of application. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

plots were used to visualize DEGs, separate for each treatment dose. Gene sets in the molecular functions ontology of Gene Ontology (GO) database were used to assess gene set enrichment [30]. *clusterProfiler* package was used to carry out gene set enrichment analysis [31]. Calculation of semantic similarity between gene sets was carried out using *revigo* package in R [32]. Calculation parameter were set to c = 0.5, species = Homo Sapiens, and semantic similarity measure of Lin was used. GO Terms were plotted as scatter plot and sorted by the value of dispensability. R language (R Foundation for Statistical Computing, Vienna, Austria, version 4.1.2) on the R Studio environment was used to compute statistical analyses and visualize data. All statistical analyses were conducted two-sided.

3. Results

A set of n = 1861 FDA-approved compounds was tested *in vitro* in an attempt to identify commonly used drugs and related compounds with relevant interfering effects on the expression of hepatic SELENOP. The systematic screening was conducted 48 h after applying 10 μmol/L of compound (f.c.) in DMSO (0.5% v/v, f.c.) by quantification of extracellular SELENOP concentrations in cell culture medium (Fig. 1). Applying an initial fold-change threshold of <0.5 and >2.0, a total of 236 modulators of hepatic SELENOP expression were identified (Fig. 1A). Replication of the findings were conducted in triplicates with a parallel assessment of cell viability (Fig. 1B). None of the substances with apparent positive effects in the initial assessment displayed an effect of >2.0 during the verification. Two criteria were applied to further

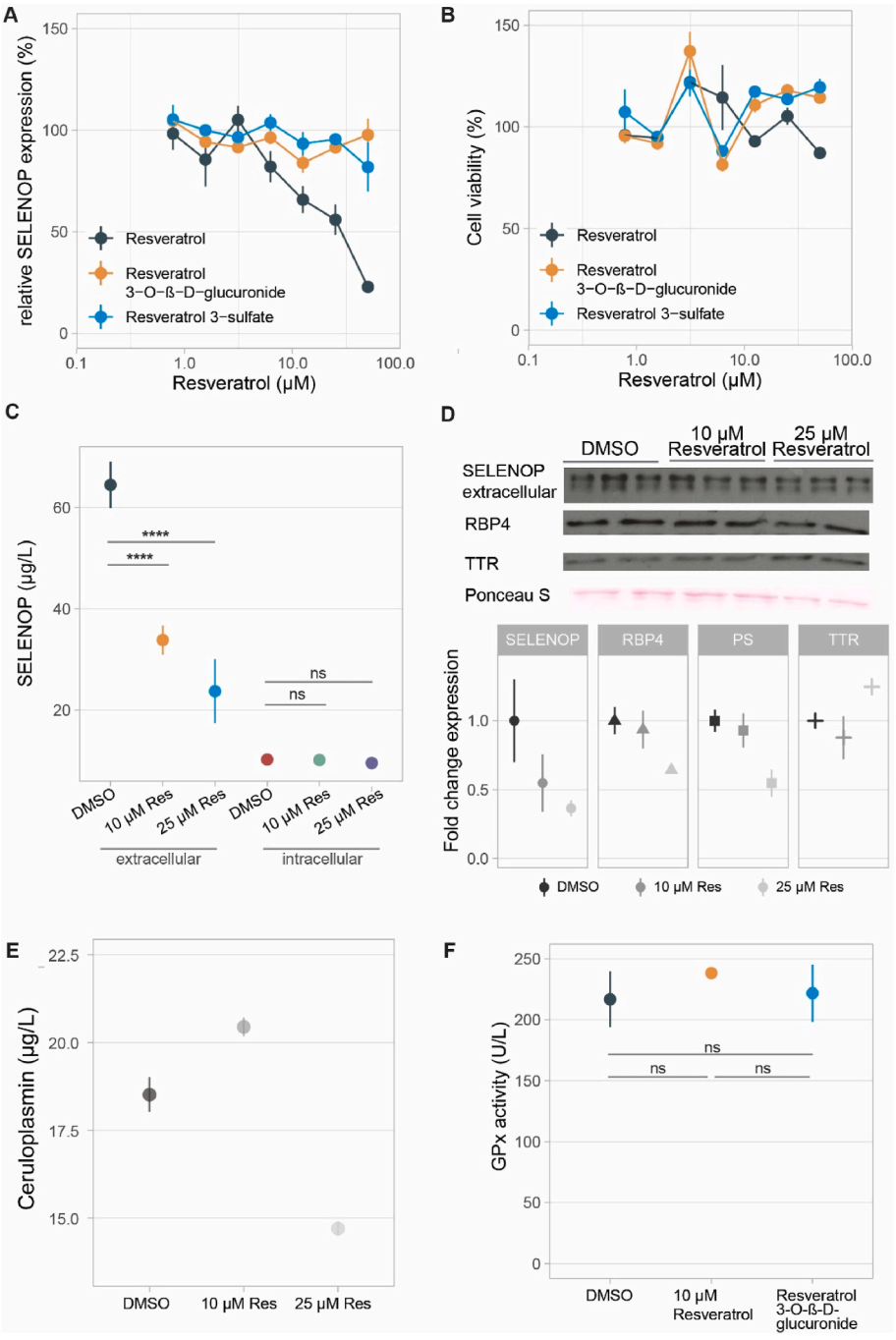


Fig. 2. Resveratrol but not its main metabolites reduces SELENOP expression in HepG2 cells. (**A**) SELENOP expression was reduced in a concentration-depended manner by resveratrol but not by its metabolites (n = 3). (**B**) Cell viability of HepG2 cells was not affected by resveratrol (Res) or its metabolites resveratrol 3-O-β-D-glucuronide or resveratrol 3-sulfate, in a range from 0.8 to 50.0 μmol/L (n = 3). (**C**) Extracellular SELENOP concentrations were decreased at 10 and 25 μmol/L resveratrol, but intracellular SELENOP was not affected (n = 6). (**D**) Western blot analysis of treated HepG2 cells with 10 or 25 μmol/L resveratrol for 48 h showed a dose-dependent reduction in extracellular SELENOP in comparison to control (DMSO). In parallel, RBP4 and albumin (PS, Ponceau stain) decreased upon exposure to 25 μM, while TTR increased, and (**E**) Ceruloplasmin concentrations decreased. (**F**) Treatment with 10 μmol/L resveratrol or resveratrol 3-O-ß-D-glucuronide for 48 h did not alter GPx activity in the cell lysates. Comparisons between groups were conducted by Wilcoxon rank-sum test. *p*-values <0.05 were considered statistically significant; n. s. indicates $p \geq 0.05$; **** indicates $p < 0.0001$.

narrow down the number of the most relevant interfering substances, namely an effect size of more than three-fold (final SELENOP concentration of <35% as compared to DMSO control) without eliciting toxicity (final mean cell viability of >90%). From all the substances, resveratrol, vidofludimus and antimony potassium tartrate fulfilled the criteria and qualified as efficient negative modulators of SELENOP expression (Fig. 1B&C).

Categorizing the group of verified active substances (n = 38) according to their primary field of application, more than half of the drugs are related to the treatment of malignant diseases, whereas the remainder of the substances belong to the group of immunosuppressants, therapeutics for infectious diseases, dietary supplements or belong to other more specific categories (Fig. 1D&E). The candidates known from prior work, i.e., aminoglycosides and metformin, caused a relative moderate suppression of SELENOP only (Supplementary Table 1). Among the statins, a heterogeneous picture across the different statin compounds emerged, with some toxic effects (e.g. pitavastatin and simvastatin) and a consistent suppression of SELENOP in particular by fluvastatin and atorvastatin. A detailed overview on all the drugs, their efficiency of SELENOP suppression, and the corresponding effects on cell viability are provided (**Supplementary Excel File 1)**.

Due to the fact that vidofludimus and antimony potassium tartrate have a rather limited area of application, additional detailed analyses were conducted with resveratrol. First, the dose-dependency of the effects of resveratrol on SELENOP expression was determined. Resveratrol reduced extracellular concentrations of SELENOP in a concentration dependent manner, down to <25% of control at a concentration of 50.0 μmol/L (f.c.) (Fig. 2A). Under these conditions, cell viability was not negatively affected (Fig. 2B). The two major metabolites of resveratrol, namely the 3-O-ß-D-glucuronide and resveratrol-3-sulfate, were without effect and did neither alter extracellular SELENOP concentration nor cell viability in a concentration range from 0.8 μmol/L to 50.0 μmol/L (Fig. 2A&B). Using resveratrol at 10 μmol/L (f.c.), intracellular SELENOP was not altered as compared to control, while extracellular SELENOP concentration decreased from 64.5 ± 4.6 μg/L to 33.8 ± 2.9 μg/L ($p < 0.0001$) (Fig. 2C). Similarly, resveratrol at 25 μmol/L caused a further reduction in extracellular SELENOP to 23.7 ± 6.3 μg/L (36.7% of control, $p < 0.0001$) (Fig. 2C). These effects were confirmed by Western blot analysis (Fig. 2D). Under the same conditions, the concentration of extracellular RBP4 declined, albeit not as strongly as SELENOP, and albumin concentrations detected by Ponceau S staining showed a similar pattern of changes as observed for RBP4. Extracellular concentrations of TTR were elevated upon exposure to 25 μM resveratrol (Fig. 2D). Ceruloplasmin concentrations were elevated in response to 10 μM resveratrol, and suppressed in response to 25 μM resveratrol (Fig. 2E). Under these conditions, glutathione peroxidase (GPx) activity in the cell lysates form the treated HepG2 cells showed no significant difference in response to 10 μM resveratrol or its metabolite (Fig. 2F).

To shed light on the potential mechanism behind the effects of resveratrol on SELENOP, and identify differentially expressed genes (DEG) under these conditions, RNA sequencing with poly-A selection of treated HepG2 cells was performed. The resveratrol treatment at 10 μmol/L (Fig. 3A, Supplementary Fig. 1A) and at 25 μmol/L (Fig. 3B, Supplementary Fig. 1B) disclosed a significant downregulation of 98 and 489 genes, respectively, and a significant upregulation of 145 and 1079 genes, respectively (Fig. 3C). RNA-seq analysis of the cells treated by resveratrol at a concentrations of 25 μmol/L showed a DEG coverage of 99.4% of down regulated genes and 98.6% of upregulated genes (Fig. 3C). The FC-FC regression of DEG and non-DEG yielded a correlation coefficient of R = 0.824; $p < 0.001$ (Fig. 3D), indicating a

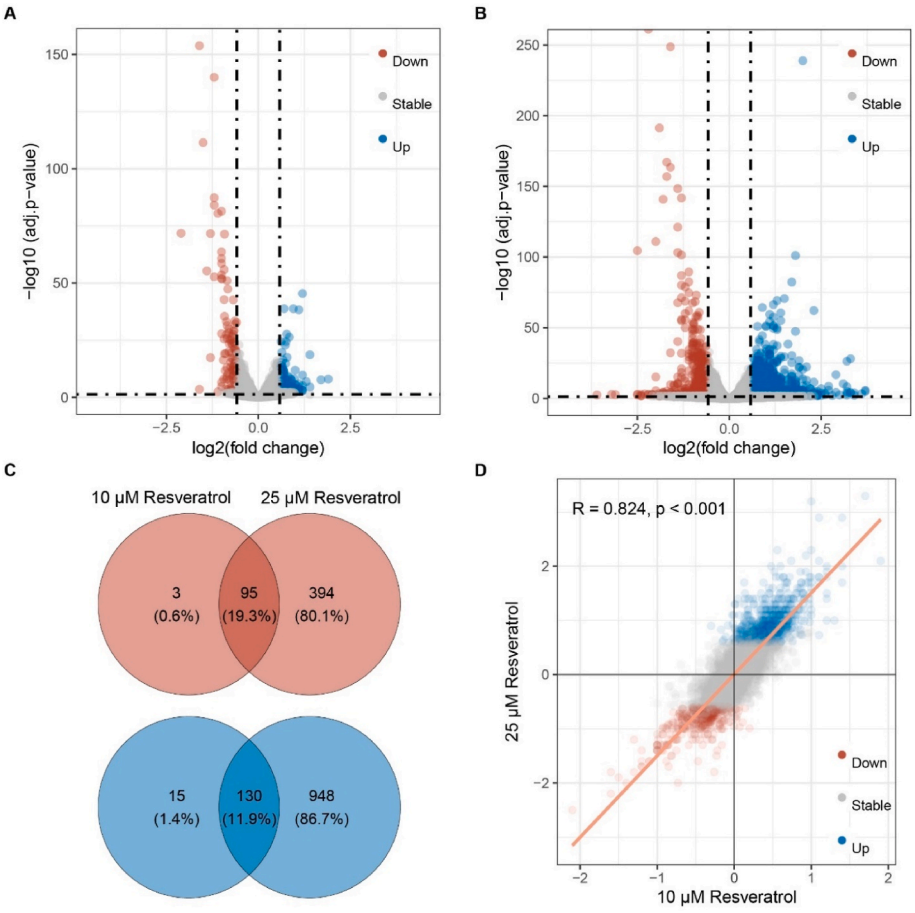


Fig. 3. Analysis of differently expressed genes (DEGs) of resveratrol treated HepG2 cells. Volcano plots with the log2 fold changes (x-axis) in gene expression for (**A**) 10 μmol/L or (**B**) 25 μmol/L resveratrol treatment and statistical significance (−log10 adjusted p value) on the y-axis. Blue circles represent down regulated genes, red circles represent up regulated genes, and dashes lines indicate cut-off values of log2(1.5) and -log2(1.5). (**C**) Comparison of fold-change of DEGs at 10 and 25 μmol/L resveratrol treatment yielded a Spearman's correlation coefficient of 0.824, $p < 0.001$. (**D**) Expression pattern of DEGs were attuned to resveratrol treatment. 99.4% of down-regulated genes and 98.6% of up-regulated genes were identified in both treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

consistent effect on gene expression in a concentration dependent manner. Resveratrol treatment of HepG2 cells resulted in no significant effect on the mRNA expression of any of the 25 human genes encoding selenoproteins or of any of the key selenoprotein-relevant transcription factors (SRY, NFAT or SP1). Within the group of key factors involved in Se metabolism and selenoprotein biosynthesis, three genes were differentially expressed, namely SCLY (log2 FC: −0.6; p adj.: 6.2×10^{-5}), SEPSECS (log2 FC: 0.7; p adj.: 8.2×10^{-11}) and SECISBP2L (log2 FC: 0.59; p adj.: 6.9×10^{-5}); Supplementary Table 2). All three genes are of particular importance for controlling selenoprotein expression patterns and Se-dependent pathology, as shown in specifically targeted animal models [33–35].

In order to reduce redundancy in the gene set enrichment analysis, the calculation of semantic similarity measures between gene ontology (GO) terms was calculated (Fig. 4). The top six overrepresented GO terms correlating with decreased SELENOP concentration were related to GO:0004553 (hydrolase activity and hydrolyzing O-glycosyl compounds), to GO:0005215 (transporter activity), to GO:0005509 (calcium ion binding), to GO:0008093 (cytoskeletal anchor activity), to GO:0008569 (minus-end-directed microtubule motor activity), and to GO:0046873 (metal ion transmembrane transporter activity) (Fig. 4A). The top six inverse correlated GO terms were related to GO:0003735 (structural constituent of ribosome), to GO:0005198 (structural molecule activity), to GO:0008574 (plus-end-directed microtubule motor activity), to GO:0009055 (electron transfer activity), to GO:0031386 (protein tag), and to GO:0046982 (protein heterodimerization activity) (Fig. 4B).

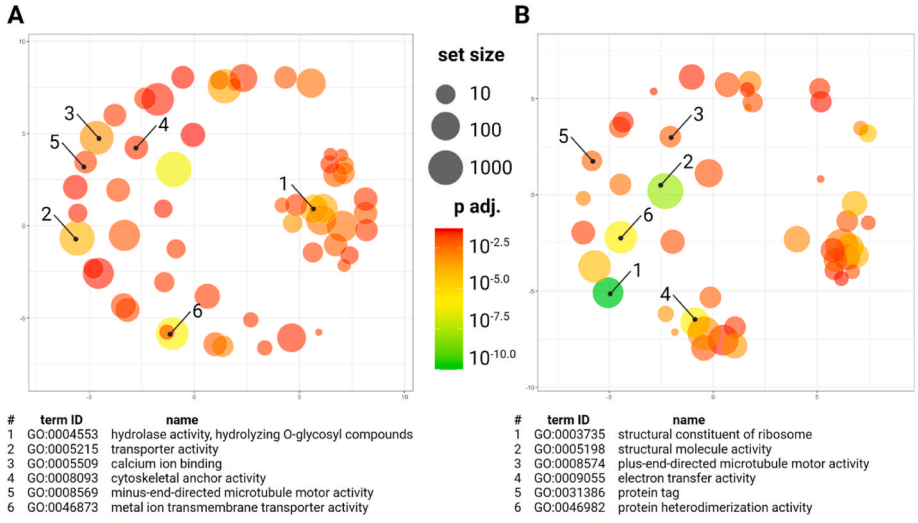
4. Discussion

The hepatic transport protein SELENOP is essential for the distribution of the trace element Se within the organism to the sites of need, especially in times of Se deficiency [10,11,36]. Uptake at privileged target tissues is tightly controlled and mediated by the lipoprotein receptors LRP2 (megalin) and LRP8 (ApoER2), respectively [37,38]. In serum, SELENOP accounts for 39%–61% of circulating Se, with the remainder mainly due to extracellular glutathione peroxidase (GPx3) and other Se-containing proteins, in particular SeMet-containing albumin [39–41]. Se deficiency may result from insufficient supply [42], mutations in genes with key importance for selenoprotein biosynthesis [43], disease [12] or from gene disruption in model systems [36]. Targeted deletion of SELENOP expression in mice has indicated its essential role for regular development under limiting Se supply, causing restricted growth, male infertility, and severe neurological defects [44–49]. The severe phenotype has been recently verified in a dog model of SELENOP loss [50]. A disruption of regular SELENOP biosynthesis and secretion therefore constitutes a relevant health risk.

Endogenous and external factors have been identified that modulate SELENOP expression, yet a systematic examination of pharmacological drugs and other substances was missing. This study presents an experimental strategy to conduct such a screening, using HepG2 cells as a robust human cell line known for a relatively high and modifiable SELENOP expression level [51–54]. Using a cut-off for the effect size of at least 2-fold, a total of 236 substances out of 1861 FDA approved drugs (12.7%) were identified as potentially relevant modulators, the majority of which suppressing extracellular SELENOP concentrations. Applying more stringent criteria considering both viability of the cells and an effect size of at least three-fold, the number of hits was reduced to three paradigmatic compounds, namely vidofludimus, antimony potassium tartrate and resveratrol.

Vidofludimus is a second-generation inhibitor of dihydroorotate dehydrogenase and a novel candidate for the treatment of a variety of immune-related diseases by inhibiting the proliferation of activated T and B cells [55]. It is characterized by a positive safety profile with some efficiency in patients with rheumatoid arthritis [56] or multiple sclerosis [57]. Strong effects have been observed in rodent models of non-alcoholic fatty liver disease (NAFLD), where vidofludimus was identified as a potent modulator of farnesoid X receptor, reducing dextran sulfate-induced colitis [58]. In a small human intervention trial, vidofludimus proved of some efficiency in primary sclerosing cholangitis without displaying drug-related adverse events [59]. These results would be compatible with an interference of SELENOP secretion, as loss of the essential trace element from liver into the circulation would be minimized, raising intracellular Se availability in hepatocytes for biosynthesis of protective selenoproteins supporting treatment. However, in case the present *in vitro* findings were relevant in human physiology, long-term application of vidofludimus might impair systemic Se transport, with potential adverse effects in target tissues like brain or endocrine glands [12,60].

The application of antimony-containing drugs has a long history in traditional and anthroposophic medicine to address tropical diseases, in particular in the treatment of leishmaniasis and schistosomiasis [61]. In an attempt of repurposing existing drugs for chemotherapy, antimony potassium tartrate was identified to inhibit several receptor tyrosine kinases, impair tumour angiogenesis and tumour growth in a mouse model of lung cancer [62]. In addition, antimony can induce apoptosis in lymphoid tumour cells by enhanced production of reactive oxygen species and associated loss of mitochondrial potential [63]. Positive effects of antimony compounds were also reported on keratinocytes, highlighting a potential suitability for the topical treatment of mild



#	term ID	name
1	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds
2	GO:0005215	transporter activity
3	GO:0005509	calcium ion binding
4	GO:0008093	cytoskeletal anchor activity
5	GO:0008569	minus-end-directed microtubule motor activity
6	GO:0046873	metal ion transmembrane transporter activity

#	term ID	name
1	GO:0003735	structural constituent of ribosome
2	GO:0005198	structural molecule activity
3	GO:0008574	plus-end-directed microtubule motor activity
4	GO:0009055	electron transfer activity
5	GO:0031386	protein tag
6	GO:0046982	protein heterodimerization activity

Fig. 4. Differentially expressed biological processes in HepG2 cells treated with 25 μmol/L resveratrol. (**A**) Overrepresented and (**B**) non-overrepresented GO terms underwent the clustering algorithm analysis REVIGO to reduce redundancy. Top six GO Terms are labeled with numbers. The dot size indicates the number of genes in each gene set. Dot color indicates adjusted p-values form gene set enrichment analysis. Statistical significance is color coded: red equals p adj. = 0.05, yellow equals p adj. = 1×10^{-6} and green equals p adj. = 1×10^{-10}. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

forms of psoriasis [64]. Severe side effects have been reported from experimental animal studies, where reactive oxygen species increased upon antimony exposure along with elevated autophagy and suppressed Akt/mTOR activity, finally causing neuronal apoptosis [65]. Collectively, antimony compounds must be classified as toxic substances, inducing an oxidative dysbalance and cell death at higher concentrations, with potential benefits upon low dose exposure and under short course regimens [66]. Again, a concurrent suppression of SELENOP secretion from liver would positively increase bactericidal and anti-virus activities in the circulation that are elicited via an increased tone of reactive oxygen species, and long-term applications may necessitate a monitoring of Se status and potential supplementation efforts to avoid systemic deficiency.

The polyphenol resveratrol emerged as the third and most prominent potent negative modulator of hepatic SELENOP expression, causing two-fold reduced SELENOP levels in the cell culture medium at an exposure concentration of 25 μmol/L. Resveratrol is an over the counter (OTC) drug that is also available as a nutritional supplement, and occurs naturally in grape skin, seeds, wine and berries in varying amounts [67,68]. Its beneficial effects on human health have been intensively studied and discussed since decades [69–72], including efficient protection from tumorigenesis, cardiovascular disease, neurodegeneration, and supporting wound healing [73–76]. Collectively, the effects of resveratrol along with other plant-derived polyphenols e.g. quercetin, procyanidin C1 or catechin are manifold, and include senolytic activities, i.e., enable the clearing of inflammation- and cancer-promoting senescent cells in the ageing organism and thereby extending lifespan [77,78]. The additional metabolic benefits are ascribed mainly to a positive modulation of the family of sirtuins [79,80], and by reducing intracellular H_2O_2 concentration [81]. In addition, proteins involved in nitric oxide and insulin signalling, and certain nuclear hormone receptors, such as PPAR-gamma, are identified as direct targets of resveratrol action [82,83]. A better understanding of the concentrations needed and synergistic interactions with other senolytic drugs holds great therapeutic promise [84].

Typical intakes in human subjects are in the range of 500 mg resveratrol per day, leading to serum peak concentrations of the unmodified compound of 71.2 ± 42.4 μg/L, i.e. 0.3 ± 0.2 μmol/L, with a serum half-life of 5.1 h [85]. While this concentration is below the range studied in the present analysis, the direct comparison of serum and local hepatic concentrations may be misleading as the nutritional intake of resveratrol will first yield relatively high local concentrations in liver due to the direct supply via the portal vein, accumulation processes in hepatocytes and enterohepatic cycling [86]. Accordingly, health effects from supplemental resveratrol are consistently observed in models of liver injury, where the stilbenoid accumulates [87,88]. Consecutively, a majority of resveratrol becomes then conjugated with sulphates or glucuronides by hepatic biotransformation yielding a range of secondary metabolites. Interestingly, our study indicated that neither resveratrol 3-O-β-D-glucuronide nor resveratrol 3-sulfate had modulating effects on hepatic SELENOP expression, highlighting high specificity of the effect for the unmodified resveratrol. Suppressed hepatic SELENOP expression would again negatively affect systemic Se supply, causing an exposure of most sensitive tissues to elevated oxidative stress, e.g. the brain [89], kidneys [90], immune cells [91] or endocrine tissues [92]. Consequently, an impaired SELENOP secretion would shift the trace element balance in favor of hepatocytes and at the expense of target tissues with potential benefits in the short run, but considerable and growing health risks from Se deficiency for SELENOP-dependent tissues upon chronic exposure. Again, a close monitoring of subjects actively taking pharmacological dosages of resveratrol is necessary in order to better understand the potential side effects on Se metabolism, and whether the findings reported in this study may be of relevance for Se-dependent health risks [1,2].

The exposure to resveratrol affects a large number of direct and indirect target genes and several liver-derived serum proteins. Our analysis indicated a suppressing effect on extracellular concentrations of RBP4, CP and albumin, albeit not yet at 10 μM but at the high exposure level of 25 μM only. In this respect, SELENOP secretion appears more sensitive to resveratrol, thereby indicating some specificity of the effects. This notion is further underlined by the effects observed on TTR, where a stimulating effect on extracellular levels is detected at 25 μM resveratrol. Collectively, the comparison of the five secreted transporters analyzed in our study (albumin, CP, RBP4, SELENOP and TTR) highlights protein-specific effects, likely due to a varying sensitivity of different secretory vesicles to the modulating effects of resveratrol on movement, membrane fusion and content release. This interpretation is in line with other hepatic vesicle-associated proteins, like the insulin receptor, glucose transporter GLUT4, or the LDL-receptors [93]. Given that SELENOP is specifically recognized and bound by members of the low-density lipoprotein receptor-related protein (LRP) family, namely LRP1, LRP2 and LRP8 [11], it is conceivable that its transport and secretion may be controlled via specific retention and release mechanisms involving certain subsets of secretory vesicles. This notion deserves to be experimentally studied in order to gain a better understanding of SELENOP trafficking, its release and hepatic Se metabolism.

Accordingly, among the large number of molecular targets affected by resveratrol exposure, several genes and molecular processes related to ion transport, cytoskeleton formation and in particular to microtubule activity were among the top over- and underrepresented GO terms. Particularly the downregulation of plus-end directed microtubule motor activity, and simultaneously the inverse upregulation of minus-end directed activity argue for a direct effect on the intracellular cargo transportation to the plasma membrane [94,95]. These combined effects may impair the intracellular traffic of certain vesicles and affect cargo transport. This interpretation is supported by a lack of effects of resveratrol on SELENOP mRNA concentrations or other selenoprotein genes. Resveratrol has been reported to affect vesicle trafficking before, in particular in adipocytes and hepatocytes [96,97]. Direct effects on SIRT1-dependent autophagy in liver have been described, positively affecting ER stress and in favor of resolving steatosis [98].

Beside resveratrol, we have identified a second relevant and common dietary constituent, namely ellagic acid, abundant in many fruits and vegetables [99]. Its effect on SELENOP expression would synergize with resveratrol, and may be of specific relevance for a vegetarian diet, that is known to be relatively low in Se supply in Europe anyhow [42,100]. Among other compounds with considerable effects on SELENOP expression are several commonly used drugs such as the immunosuppressant mycophenolic acid. The negative effect observed is compatible to clinical studies, where mycophenolic acid suppressed serum Se concentrations in female renal transplant recipients [101].

Surprisingly, anti-cancer drugs made up the most frequent category of the compounds identified (53%, i.e., 20/38). Some of these drugs efficiently suppressed SELENOP despite relatively moderate effects on cell viability only, including common therapeutic agents in breast cancer like capecitabine, gemcitabine or abemaciclib [102,103]. This finding deserves particular attention when considering the recent findings on the inverse dose-dependent relation between serum Se status biomarkers and breast cancer recurrence and death [104]. In how far an adjuvant Se supplementation may support the immune system and survival under such adverse chemotherapeutic drug exposure needs to be determined in clinical studies, but drug effects on systemic Se transport would constitute a relevant but readily addressable health issue [60,105]. The findings may most notably concern individuals with an already low Se status, e.g. due to inadequate supply, genetic predisposition or disease-driven deficiency, as known from injury, sepsis, cancer or severe COVID-19 [106,107]. Particularly under such conditions, a low Se status is associated with mortality risk, hence further drug-mediated impairment of Se transport, even to a small degree, may further exacerbate the clinical phenotype.

Overall, the high frequency of substances with notable, negative

effects on SELENOP, and therefore on Se distribution, underlines the necessity to evaluate treatments and combinations of drugs on their potential effects on the Se status of the patients. While the discussion on potential physiological consequences includes a high range of uncertainty by extrapolation into the intact organism, it still points out the potential of individual drugs and drug combinations to aggravate and contribute to Se-deficiency.

Our study has several strengths, including the unbiased, systematic approach to identify potent regulators of SELENOP expression, and the relatively large set of well-characterized substances that have been evaluated and are mostly of clinical relevance. The standardized screening protocol provides good opportunities for future research to identify specific modifiers of hepatic SELENOP expression, alone and in combinations, with relevant consequences for avoiding side effects, maintaining health and supporting recovery from disease.

Among the notable limitations of our study is the moderate robustness of the initial screening, which is a common limitation in high throughput analyses. Although we have observed similar results for most of the hits when we re-screened the drugs in triplicates, the main screening result was recorded from a single measurement. Secondly, we have selected and used a single cancer cell line to conduct the screening and to validate our results. There are several advantages of the HepG2 cells used, including the reliable, sensitive, dynamic and relatively high expression of SELENOP and other secreted proteins, however, generalizability of the results still needs to be proven in additional model systems.

Data and code availability

Data on differentially expressed genes generated in RNA-seq analyses are downloadable from the supplementary material. No custom code was generated for the purpose of this study.

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Author contributions

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Declaration of competing interest

L.S. holds shares of selenOmed GmbH, a company involved in Se status assessment. The other authors declare no competing interests.

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Appendix A. Supplementary data

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References

[1] L.V. Papp, A. Holmgren, K.K. Khanna, Selenium and selenoproteins in health and disease, Antioxidants Redox Signal. 12 (7) (2010) 793–795.
[2] M.P. Rayman, Selenium and human health, Lancet 379 (9822) (2012) 1256–1268.
[3] V.M. Labunskyy, D.L. Hatfield, V.N. Gladyshev, Selenoproteins: molecular pathways and physiological roles, Physiol. Rev. 94 (3) (2014) 739–777.
[4] R.F. Burk, et al., Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial, Cancer Epidemiol. Biomark. Prev. 15 (4) (2006) 804–810.
[5] T. Hilal, et al., Structure of the mammalian ribosome as it decodes the selenocysteine UGA codon, Science 376 (6599) (2022) 1338–+.
[6] M.A. Motsenbocker, A.L. Tappel, A selenocysteine-containing selenium-transport protein in rat plasma, Biochim. Biophys. Acta 719 (1) (1982) 147–153.
[7] M.P. Rayman, P. Bode, C.W. Redman, Low selenium status is associated with the occurrence of the pregnancy disease preeclampsia in women from the United Kingdom, Am. J. Obstet. Gynecol. 189 (5) (2003) 1343–1349.
[8] W.B. Minich, Selenium metabolism and biosynthesis of selenoproteins in the human body, Biochemistry (Mosc.) 87 (1) (2022). S168-s102.
[9] Y. Xia, et al., Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects, Am. J. Clin. Nutr. 92 (3) (2010) 525–531.
[10] R.F. Burk, K.E. Hill, Regulation of selenium metabolism and transport, Annu. Rev. Nutr. 35 (2015) 109–134.
[11] Y. Saito, Selenium transport mechanism via selenoprotein P-its physiological role and related diseases, Front. Nutr. 8 (2021), 685517.
[12] L. Schomburg, Selenoprotein P - selenium transport protein, enzyme and biomarker of selenium status, Free Radic. Biol. Med. 191 (2022) 150–163.
[13] M. Gupta, P.R. Copeland, Functional analysis of the interplay between translation termination, selenocysteine codon context, and selenocysteine insertion sequence-binding protein 2, J. Biol. Chem. 282 (51) (2007) 36797–36807.
[14] S. Hybsier, et al., Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P, Redox Biol. 11 (2017) 403–414.
[15] J. Martitz, et al., Factors impacting the aminoglycoside-induced UGA stop codon readthrough in selenoprotein translation, J. Trace Elem. Med. Biol. 37 (2016) 104–110.
[16] D.E. Handy, et al., Aminoglycosides decrease glutathione peroxidase-1 activity by interfering with selenocysteine incorporation, J. Biol. Chem. 281 (6) (2006) 3382–3388.
[17] A.M. Diamond, et al., Lovastatin effects on human breast carcinoma cells. Differential toxicity of an adriamycin-resistant derivative and influence on selenocysteine tRNAS, Biochem. Mol. Biol. Int. 38 (2) (1996) 345–355.
[18] H. Takayama, et al., Metformin suppresses expression of the selenoprotein P gene via an AMP-activated kinase (AMPK)/FoxO3a pathway in H4IIEC3 hepatocytes, J. Biol. Chem. 289 (1) (2014) 335–345.
[19] X. Yao, et al., Cyclosporine A downregulates selenoprotein P expression via a signal transducer and activator of transcription 3-forkhead box protein O1 pathway in hepatocytes in vitro, J. Pharmacol. Exp. Therapeut. 382 (2) (2022) 199–207.
[20] J.J. Klemens, et al., Antioxidant enzyme levels inversely covary with hearing loss after amikacin treatment, J. Am. Acad. Audiol. 14 (3) (2003) 134–143.
[21] B. Moosmann, C. Behl, Selenoprotein synthesis and side-effects of statins, Lancet 363 (9412) (2004) 892–894.
[22] E.D. Kantor, et al., Trends in prescription drug use among adults in the United States from 1999-2012, JAMA 314 (17) (2015) 1818–1830.
[23] C. Cacador, et al., The role of nutritional status on polypharmacy, cognition, and functional capacity of institutionalized elderly: a systematic review, Nutrients 13 (10) (2021).
[24] J. Jyrkka, et al., Polypharmacy and nutritional status in elderly people, Curr. Opin. Clin. Nutr. Metab. Care 15 (1) (2012) 1–6.
[25] M.Y. Lee, et al., Selenoproteins and the senescence-associated epitranscriptome, Exp. Biol. Med. (2022), 15353702221116592.
[26] Z. Li, et al., Ribosome stalling during selenoprotein translation exposes a ferroptosis vulnerability, Nat. Chem. Biol. 18 (7) (2022) 751–761.
[27] L. Flohé, W.A. Günzler, Assays of glutathione peroxidase, Methods Enzymol. 105 (1984) 114–121.
[28] J. Hackler, et al., Copper and selenium status as biomarkers of neonatal infections, J. Trace Elem. Med. Biol. 58 (2020), 126437.
[29] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (12) (2014) 1–21.

[30] M. Ashburner, et al., Gene Ontology: tool for the unification of biology, Nat. Genet. 25 (1) (2000) 25–29.
[31] T. Wu, et al., clusterProfiler 4.0: a universal enrichment tool for interpreting omics data, Innovation 2 (3) (2021), 100141.
[32] F. Supek, et al., REVIGO summarizes and visualizes long lists of gene ontology terms, PLoS One 6 (7) (2011), e21800.
[33] L.M. Watanabe, et al., Effect of statin treatment in obese selenium-supplemented mice lacking selenocysteine lyase, Mol. Cell. Endocrinol. 533 (2021), 111335.
[34] N. Fradejas-Villar, et al., Missense mutation in selenocysteine synthase causes cardio-respiratory failure and perinatal death in mice which can be compensated by selenium-independent GPX4, Redox Biol. 48 (2021), 102188.
[35] Z.M. Dai, et al., SECISBP2L-Mediated selenoprotein synthesis is essential for autonomous regulation of oligodendrocyte differentiation, J. Neurosci. 42 (30) (2022) 5860–5869.
[36] U. Schweizer, et al., The neurobiology of selenium: looking back and to the future, Front. Neurosci. 15 (2021), 652099.
[37] G.E. Olson, et al., Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells, J. Biol. Chem. 283 (11) (2008) 6854–6860.
[38] R.F. Burk, et al., Selenoprotein P and apolipoprotein E receptor-2 interact at the blood-brain barrier and also within the brain to maintain an essential selenium pool that protects against neurodegeneration, Faseb. J. 28 (8) (2014) 3579–3588.
[39] B. Akesson, T. Bellew, R.F. Burk, Purification of selenoprotein P from human plasma, Biochim. Biophys. Acta 1204 (2) (1994) 243–249.
[40] J.T. Deagen, et al., Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma, Anal. Biochem. 208 (1) (1993) 176–181.
[41] S. Letsiou, et al., Gender-specific distribution of selenium to serum selenoproteins: associations with total selenium levels, age, smoking, body mass index, and physical activity, Biofactors 40 (5) (2014) 524–535.
[42] M.P. Rayman, Food-chain selenium and human health: emphasis on intake, Br. J. Nutr. 100 (2) (2008) 254–268.
[43] E. Schoenmakers, K. Chatterjee, Human disorders affecting the selenocysteine incorporation pathway cause systemic selenoprotein deficiency, Antioxidants Redox Signal. 33 (7) (2020) 481–497.
[44] K.E. Hill, et al., Deletion of selenoprotein P alters distribution of selenium in the mouse, J. Biol. Chem. 278 (16) (2003) 13640–13646.
[45] L. Schomburg, et al., Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues, Biochem. J. 370 (Pt 2) (2003) 397–402.
[46] K.E. Hill, et al., The selenium-rich C-terminal domain of mouse selenoprotein P is necessary for the supply of selenium to brain and testis but not for the maintenance of whole body selenium, J. Biol. Chem. 282 (15) (2007) 10972–10980.
[47] K. Renko, et al., Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice, Biochem. J. 409 (3) (2008) 741–749.
[48] R. Pillai, J.H. Uyehara-Lock, F.P. Bellinger, Selenium and selenoprotein function in brain disorders, IUBMB Life 66 (4) (2014) 229–239.
[49] U. Schweizer, et al., Seizures, ataxia and parvalbumin-expressing interneurons respond to selenium supply in Selenop-deficient mice, Redox Biol. 57 (2022), 102490.
[50] M. Christen, et al., Deletion of the SELENOP gene leads to CNS atrophy with cerebellar ataxia in dogs, PLoS Genet. 17 (8) (2021), e1009716.
[51] C.S. Hoefig, et al., Comparison of different selenocompounds with respect to nutritional value vs. toxicity using liver cells in culture, J. Nutr. Biochem. 22 (10) (2011) 945–955.
[52] S. Tauber, et al., Activation of nrf2 by electrophiles is largely independent of the selenium status of HepG2 cells, Antioxidants 10 (2) (2021).
[53] M. Schwarz, et al., Copper interferes with selenoprotein synthesis and activity, Redox Biol. 37 (2020), 101746.
[54] Y. Lin, et al., Selenium-enriched peptides isolated from Cardamine violifolia are potent in suppressing proliferation and enhancing apoptosis of HepG2 cells, J. Food Sci. 87 (7) (2022) 3235–3247.
[55] S. Peterson, et al., Updates on efficacy and safety outcomes of new and emerging disease modifying therapies and stem cell therapy for Multiple Sclerosis: a review, Mult. Scler. Relat. Disord. 68 (2022), 104125.
[56] A. Muehler, et al., The selective oral immunomodulator vidofludimus in patients with active rheumatoid arthritis: safety results from the COMPONENT study, Drugs R 19 (4) (2019) 351–366.
[57] A. Muehler, et al., Vidofludimus calcium, a next generation DHODH inhibitor for the Treatment of relapsing -remitting multiple sclerosis, Mult. Scler. Relat. Disord. 43 (2020).
[58] Y. Zhu, et al., Repositioning an immunomodulatory drug vidofludimus as a farnesoid X receptor modulator with therapeutic effects on NAFLD, Front. Pharmacol. 11 (2020) 590.
[59] E.J. Carey, et al., A pilot study of vidofludimus calcium for treatment of primary sclerosing cholangitis, Hepatol Commun 6 (7) (2022) 1589–1597.
[60] G. Barchielli, A. Capperucci, D. Tanini, The role of selenium in pathologies: an updated review, Antioxidants 11 (2) (2022).
[61] A. Periferakis, et al., Availability, toxicology and medical significance of antimony, Int. J. Environ. Res. Publ. Health 19 (8) (2022).
[62] B. Wang, et al., The antiparasitic drug, potassium antimony tartrate, inhibits tumor angiogenesis and tumor growth in nonsmall-cell lung cancer, J. Pharmacol. Exp. Therapeut. 352 (1) (2015) 129–138.
[63] V. Lecureur, et al., Potassium antimonyl tartrate induces caspase- and reactive oxygen species-dependent apoptosis in lymphoid tumoral cells, Br. J. Haematol. 119 (3) (2002) 608–615.
[64] F. Gendrisch, et al., Anti-psoriatic effects of antimony compounds in vitro, Molecules 26 (19) (2021).
[65] X. Wang, et al., Antimony, a novel nerve poison, triggers neuronal autophagic death via reactive oxygen species-mediated inhibition of the protein kinase B/mammalian target of rapamycin pathway, Int. J. Biochem. Cell Biol. 114 (2019), 105561.
[66] S. Sundar, J. Chakravarty, Antimony toxicity, Int. J. Environ. Res. Publ. Health 7 (12) (2010) 4267–4277.
[67] A. Shrikanta, A. Kumar, V. Govindaswamy, Resveratrol content and antioxidant properties of underutilized fruits, J. Food Sci. Technol. 52 (1) (2015) 383–390.
[68] R.A. Bohara, et al., Recent overview of resveratrol's beneficial effects and its nano-delivery systems, Molecules 27 (16) (2022).
[69] M. Jang, et al., Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (5297) (1997) 218–220.
[70] E.N. Frankel, A.L. Waterhouse, J.E. Kinsella, Inhibition of human LDL oxidation by resveratrol, Lancet 341 (8852) (1993) 1103–1104.
[71] S.J. Park, et al., Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases, Cell 148 (3) (2012) 421–433.
[72] S. Timmers, et al., Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans, Cell Metabol. 14 (5) (2011) 612–622.
[73] E. Lazzarini, et al., Stress-induced premature senescence is associated with a prolonged QT interval and recapitulates features of cardiac aging, Theranostics 12 (11) (2022) 5237–5257.
[74] M. Alavi, et al., Resveratrol mediates its anti-cancer effects by Nrf2 signaling pathway activation, Cancer Cell Int. 21 (1) (2021) 579.
[75] A. Hecker, et al., The impact of resveratrol on skin wound healing, scarring, and aging, Int. Wound J. 19 (1) (2022) 9–28.
[76] M.G. Dos Santos, et al., Neuroprotective effects of resveratrol in in vivo and in vitro experimental models of Parkinson's disease: a systematic review, Neurotox. Res. 40 (1) (2022) 319–345.
[77] M.J. Yousefzadeh, et al., Fisetin is a senotherapeutic that extends health and lifespan, EBioMedicine 36 (2018) 18–28.
[78] Q. Xu, et al., The flavonoid procyanidin C1 has senotherapeutic activity and increases lifespan in mice, Nat Metab 3 (12) (2021) 1706–1726.
[79] D.S. Mohar, S. Malik, The sirtuin system: the holy grail of resveratrol? J. Clin. Exp. Cardiol. 3 (11) (2012).
[80] R. Furuuchi, et al., Endothelial SIRT-1 has a critical role in the maintenance of capillarization in brown adipose tissue, iScience 25 (11) (2022), 105424.
[81] Y. Pan, et al., Resveratrol exerts antioxidant effects by activating SIRT2 to deacetylate Prx1, Biochemistry 56 (48) (2017) 6325–6328.
[82] R.H.X. Wong, P.R.C. Howe, Resveratrol counteracts insulin resistance-potential role of the circulation, Nutrients 10 (9) (2018).
[83] T.M. Barber, et al., Implications of resveratrol in obesity and insulin resistance: a state-of-the-art review, Nutrients 14 (14) (2022).
[84] A.K. Palmer, T. Tchkonia, J.L. Kirkland, Targeting cellular senescence in metabolic disease, Mol. Metabol. 66 (2022), 101601.
[85] C. Sergides, et al., Bioavailability and safety study of resveratrol 500 mg tablets in healthy male and female volunteers, Exp. Ther. Med. 11 (1) (2016) 164–170.
[86] C.H. Cottart, et al., Resveratrol bioavailability and toxicity in humans, Mol. Nutr. Food Res. 54 (1) (2010) 7–16.
[87] A. Wolf, et al., Resveratrol therapy improves liver function via estrogen-receptors after hemorrhagic shock in rats, PLoS One 17 (10) (2022), e0275632.
[88] M. Di Pascoli, et al., Resveratrol improves intrahepatic endothelial dysfunction and reduces hepatic fibrosis and portal pressure in cirrhotic rats, J. Hepatol. 58 (5) (2013) 904–910.
[89] R.F. Burk, et al., Deletion of apolipoprotein E receptor-2 in mice lowers brain selenium and causes severe neurological dysfunction and death when a low-selenium diet is fed, J. Neurosci. 27 (23) (2007) 6207–6211.
[90] U. Schweizer, et al., Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply, Biochem. J. 386 (2) (2005) 221–226.
[91] P. Li, et al., Glutathione peroxidase 4-regulated neutrophil ferroptosis induces systemic autoimmunity, Nat. Immunol. 22 (9) (2021) 1107–1117.
[92] N. Kitabayashi, et al., Role of selenoprotein P expression in the function of pancreatic β cells: prevention of ferroptosis-like cell death and stress-induced nascent granule degradation, Free Radic. Biol. Med. 183 (2022) 89–103.
[93] J. Gilleron, A. Zeigerer, Endosomal trafficking in metabolic homeostasis and diseases, Nat. Rev. Endocrinol. 19 (1) (2022) 28–45, https://doi.org/10.1038/s41574-022-00737-9.
[94] R.J. Schulze, et al., The cell biology of the hepatocyte: a membrane trafficking machine, J. Cell Biol. 218 (7) (2019) 2096–2112.
[95] S.E. Cason, E.L.F. Holzbaur, Selective motor activation in organelle transport along axons, Nat. Rev. Mol. Cell Biol. 23 (11) (2022) 699–714.
[96] A. Rezaei Farimani, et al., Effect of resveratrol supplementation on the SNARE proteins expression in adipose tissue of streptozotocin-nicotinamide induced type 2 diabetic rats, Iran. J. Med. Sci. 40 (3) (2015) 248–255.
[97] B.B. Kang, B.H. Chiang, A novel phenolic formulation for treating hepatic and peripheral insulin resistance by regulating GLUT4-mediated glucose uptake, J Tradit Complement Med 12 (2) (2022) 195–205.
[98] S. Ding, et al., Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats, PLoS One 12 (8) (2017), e0183541.
[99] D.A. Vattem, K. Shetty, Biological functionality of ellagic acid: a review, J. Food Biochem. 29 (3) (2005) 234–266.
[100] J. Hoeflich, et al., The choice of biomarkers determines the selenium status in young German vegans and vegetarians, Br. J. Nutr. 104 (11) (2010) 1601–1604.

[101] A. Wilk, et al., Serum selenium, iron, zinc, and copper concentrations in renal transplant recipients treated with mycophenolate mofetil, Biol. Trace Elem. Res. 198 (2) (2020) 371–379.
[102] A. Malmström, et al., Gemcitabine and capecitabine in combination for advanced anthracycline and taxane pre-treated breast cancer patients: a phase II study, Acta Oncol. 49 (1) (2010) 35–41.
[103] G.W. Sledge Jr., et al., The effect of abemaciclib plus fulvestrant on overall survival in hormone receptor–positive, ERBB2-negative breast cancer that progressed on endocrine therapy—monarch 2: a randomized clinical trial, JAMA Oncol. 6 (1) (2020) 116–124.
[104] K. Demircan, et al., Serum selenium, selenoprotein P and glutathione peroxidase 3 as predictors of mortality and recurrence following breast cancer diagnosis: a multicentre cohort study, Redox Biol. 47 (2021), 102145.
[105] W. Hu, et al., Food sources of selenium and its relationship with chronic diseases, Nutrients 13 (5) (2021).
[106] K. Demircan, et al., Association of COVID-19 mortality with serum selenium, zinc and copper: six observational studies across Europe, Front. Immunol. 13 (2022), 1022673.
[107] B. Flowers, A. Poles, I. Kastrati, Selenium and breast cancer - an update of clinical and epidemiological data, Arch. Biochem. Biophys. 732 (2022), 109465.