Microchemical Journal 199 (2024) 110033

Contents lists available at ScienceDirect

Microchemical Journal

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

Heteroatom-tagged proteomics of lung cancer and chronic obstructive pulmonary disease human serum reveal alterations in selenoproteins

Belén Callejón-Leblic [a,1], Saida Sánchez Espirilla [a,b,1], Carolina Gotera-Rivera [c], Rafael Santana [c], Isabel Díaz-Olivares [d], José María Marín Trigo [e], Ciro Casanova Macario [f], Borja G. Cosio [g], Antonia Fuster [h], Ingrid Solanes García [i], Juan P. de-Torres [j], Nuria Feu Collado [k], Carlos Cabrera Lopez [l], Carlos Amado Diago [m], Amparo Romero Plaza [n], Luis Alejandro Padrón Fraysse [o], Eduardo Márquez Martín [p], Margarit Marín Royo [q], Eva Balcells Vilarnau [r], Antonia Llunell Casanovas [s], Cristina Martínez González [t], Juan Bautista Galdíz Iturri [u], Celia Lacárcel Bautista [v], José Luis Gómez-Ariza [a], Antonio Pereira-Vega [o], Luis Seijo [w], José Luis López-Campos [x,y], Germán Peces-Barba [c,*], Tamara García-Barrera [a,*]

[a] *University of Huelva, Department of Chemistry, Research Center for Natural Resources, Health and the Environment (RENSMA), Faculty of Experimental Sciences, University of Huelva, Campus El Carmen, Fuerzas Armadas Ave., 21007 Huelva, Spain*
[b] *Department of Chemistry, National University of San Antonio Abad of Cusco, La Cultura Ave., 773 Cusco, Peru*
[c] *IIS-Jiménez Díaz Foundation, ISCIII-CIBERES, Madrid, Spain*
[d] *Beturia Andalusian Foundation for Health Research (FABIS), Huelva, Spain*
[e] *Hospital Miguel Servet-IISAragon, ISCIII-CIBERES, Zaragoza, Spain*
[f] *Pulmonary Department-Research Unit, Hospital Universitario Nuestra Señora de Candelaria, CIBERES, ISCIII, Universidad de La Laguna, Tenerife, Spain*
[g] *Hospital Son Espases, IdISBa, ISCIII-CIBERES., Palma De Mallorca, Spain*
[h] *Hospital Son Llàtzer, Palma De Mallorca, Spain*
[i] *Hospital Santa Creu i Sant Pau, Barcelona, Spain*
[j] *Clinica Universidad de Navarra, Pamplona, Spain*
[k] *Hospital Reina Sofía, Instituto Maimónides de Investigación Biomédica de Córdoba, Córdoba, Spain*
[l] *Hospital Doctor Negrín, Las Palmas De Gran Canarias, Spain*
[m] *Hospital Marqués de Valdecilla, Santander, Spain*
[n] *Hospital de Manacor, Manacor, Spain*
[o] *Pneumology Area of the Juan Ramón Jiménez Hospital, Huelva, Spain*
[p] *Hospital Virgen del Rocío, Instituto de Biomedicina de Sevilla (IBiS), ISCIII-CIBERES, Sevilla, Spain*
[q] *Hospital de Castellón, Castellón, Spain*
[r] *Hospital del Mar, ISCIII-CIBERES, Barcelona, Spain*
[s] *Hospital de Terrassa, Barcelona, Spain*
[t] *Hospital Central de Asturias, Oviedo, Spain*
[u] *Hospital Universitario de Cruces, ISCIII-CIBERES, Baracaldo, Spain*
[v] *Hospital Universitario de Jaén, Jaén, Spain*
[w] *University of Navarra Clinic, ISCIII-CIBERES, Madrid, Spain*
[x] *Medical-Surgical Unit for Respiratory Diseases, Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital, University of Seville, Spain*
[y] *Center for Biomedical Research in Respiratory Diseases Network (CIBERES), Carlos III Health Institute, Madrid, Spain*

Abbreviations: AF, Affinity Chromatography; AUC, Area under the curve; COPD, Chronic Obstructive Pulmonary Disease; COPD-LC, COPD patients who developed LC; COPD-vs, Very severe COPD; COPD-s, Severe COPD; COPD-mo, Moderate COPD; COPD-mi, Mild COPD; CHAiN, History Assessment in SpaiN; eGPx, Extracellular Glutathione Peroxidase; HC, Healthy Control; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; LC, Lung cancer; LOD, Limits of detection; LOQ, Limits of quantification; PCA, Principal Component Analysis; PLS-DA, Partial Least Squares–Discriminant Analysis; ROC, Receiver operating characteristic curve; SeAlb, Selenoalbumin; SEC, Size Exclusion Chromatography; SELENOP, Selenoprotein P; VIP, Variable Importance on the Projection.

* Corresponding authors.
E-mail addresses: GPeces@quironsalud.es (G. Peces-Barba), tamara@dqcm.uhu.es (T. García-Barrera).
[1] These authors contributed equally and should be considered co-first authors

https://doi.org/10.1016/j.microc.2024.110033
Received 22 September 2023; Received in revised form 17 January 2024; Accepted 22 January 2024
Available online 23 January 2024

ARTICLE INFO

Keywords:
ICP-MS
Heteroatom-tagged proteomics
Lung cancer
Chronic obstructive pulmonary disease
Selenoproteins

ABSTRACT

Heteroatom-tagged proteomics allows the absolute quantification of selenoproteins using the heteroatom as a "tag" into a selective and sensitive atomic detector instead of a molecular one. Using this analytical method, about 90% of total selenium in human serum/plasma can be measured as selenoproteins and total selenometabolites and thus, the status of selenium can be determined. Herein, we determined the absolute concentration of selenoproteins in human serum patients with lung cancer (LC) and chronic obstructive pulmonary disease (COPD), a competing cause of morbidity and mortality in smokers as well as an independent risk factor for LC. We conducted an observational study of 154 human serum samples obtained from LC and COPD patients with varying severity of disease, including COPD patients who developed LC during follow-up and healthy controls (HC). Using heteroatom-tagged proteomics, we determined extracellular glutathione peroxidase (eGPx), selenoprotein P (SELENOP), and selenoalbumin (SeAlb). Associations between selenoproteins were also studied as potential biomarkers of disease. The concentration of eGPx was significantly higher in the all-inclusive COPD cohort compared to HC, COPD patients with LC, or those with mild obstructive lung disease, while SELENOP concentration was significantly decreased in LC patients compared to HC and COPD. We found an inverse correlation between SELENOP and SeAlb in HC, but also in LC patients, and especially in patients with COPD and LC. Moreover, we found that eGPx and selenometabolite concentrations were positively associated with LC human serum. Selenoprotein concentrations were altered in COPD and LC when compared to healthy controls suggesting a potential role of the selenoproteome in the diagnosis and/or treatment of these tobacco-related diseases.

1. Introduction

The combination of inductively coupled plasma (ICP) ionization source and mass spectrometry (MS) has become a powerful tool for detecting and measuring any "heteroatoms" that occur naturally in proteins, including metals, semimetals, or biologically significant nonmetals like sulfur or phosphorus. By using high performance liquid chromatography (HPLC) or capillary electrophoresis (CE), hyphenated ICP-MS can identify chemical species and metalloproteins by utilizing the heteroatom in the biomolecule as a "tag" (heteroatom-tagged proteomics) determining the metal-containing molecule by the measurement of the element [1]. This technique allows the absolute quantification of the biomolecule without protein standards only considering the number of heteroatoms in the molecule (*e.g.* human selenoprotein P has 10 selenium atoms) [2]. This is of significant importance as protein standards are often not readily accessible, and the purity evaluation process can prove to be challenging. The hyphenated ICP-MS approach is notably more sensitive than conventional proteomic methods such as Western blot analysis, which relies on an antibody that attaches to the protein, providing only a relative quantification or quantitative polymerase chain reaction in real-time (qPCR), which solely determines the amount of a gene expressed through transcript copy number [3]. Moreover, Enzyme-Linked Immunosorbent Assay (ELISA) allows for absolute quantification, but the antigen must be well known (purified and isolated), and the precision and accuracy are poor and far from that of hyphenated ICP-MS [4].

Heteroatom-tagged proteomics has the potential to be a powerful tool for precise quantification of metalloproteins and identification of biomarkers for disease. Herein, we have developed a heteroatom-tagged proteomics method that examines the role of selenoproteins in two related diseases, such as Lung Cancer (LC) and Chronic Obstructive Pulmonary Disease (COPD).

LC is the leading cause of cancer deaths worldwide [5] while COPD is a competing cause of morbidity and mortality in smokers as well as an independent risk factor for LC, representing a major public health challenge. The burden of both diseases will likely rise in the coming decades. LC is a multifaceted disease in which elements, metabolites, selenoproteins, and even gut microbiota may have a significant role [6]. Certain elements have been linked to cancer progression [7–9] and metastasis [10,11] and their levels are significantly altered in LC human biofluids [12–15]. Selenium, for example, plays an essential role in inhibiting angiogenesis [16], improving the immune response [17], regulating the proliferation of cells [18] and antagonizing certain toxins [19]. In addition, selenium, as a key constituent of selenoproteins, has been extensively studied in LC due to its antioxidant character and chemopreventive role providing benefits for treatment as a dietary supplement [20,21]. In human serum, there exist four distinct types of selenoproteins. These include specific selenocysteine-containing selenoproteins such as glutathione peroxidase, which make up roughly 15–20 % of total selenium, as well as selenoprotein P, which accounts for over half of total selenium. In addition, there is selenoalbumin, which makes up approximately 15–20 % of total selenium a non-specifically incorporated selenium that replaces sulfur in amino acids like methionine [22,23].

The levels of selenoproteins and selenometabolites in LC human serum were suggested to be significantly altered in previous studies [21,24]. However, limited evidence has been reported on the absolute quantification of selenoproteins in LC [25–27] especially in COPD. Furthermore, we analyzed the selenoprotein profile of individuals afflicted with varying degrees of COPD, including those who developed LC during the study, in comparison to healthy subjects. Our objective was to identify potential alterations in the selenoproteins profiles that could potentially serve as biomarkers for these correlated ailments.

2. Methods

2.1. Study participants and study design

In our effort to identify changes in the concentration of selenoproteins associated with LC and COPD, we determined the concentrations of three selenoproteins (eGPx, SELENOP, and SeAlb) and the total amount of selenometabolites in 156 human serum samples obtained from 29 patients with LC, 71 with COPD, and 56 healthy controls (HC). The COPD cohort was subdivided according to disease severity as follows: COPD patients who developed LC during follow-up (COPD-LC, n = 16), very severe COPD (COPD-vs, n = 21), severe COPD (COPD-s, n = 10), moderate COPD (COPD-mo, n = 10) and mild COPD (COPD-mi, n = 33).

COPD History Assessment in SpaiN (CHAIN) is a multicenter, observational study of prospective cohorts derived from 36 Spanish hospitals. The recruitment period began on January 15, 2010, and is ongoing (Clinical Trials.gov: identifier NCT01122758). All participants signed the informed consent approved by the ethics committees of the participating centers (Hospital Universitario la Candelaria, Tenerife; Spain; IRB No. 258/2009). The cohort is active and has a follow-up of more than 10 years period with complete office visits every 12 months and telephone interviews every 6 months to evaluate exacerbations and the vital state of the subjects. The data analyzed in this study were collected from the date of recruitment until May 2015. The data were

anonymized with hierarchical access control to ensure data security. At each annual visit, information was collected on: (i) clinical aspects (socio-economic situation, anthropometric data, comorbidities, smoking, respiratory symptoms, exacerbations, quality of life, anxiety-depression scale, daily life activities, treatments); (ii) respiratory function (spirometry, blood gases, hyperinflation, diffusion, respiratory pressures); (iii) BODE index (main study variable); (iv) peripheral muscle function, and (v) blood work-up (including IgE and cardiovascular risk factors). In addition, a serum bank was created for the future determination of biomarkers, and part of the analyzed samples in this study were taken. The rest of the samples were collected at the pulmonary department of the Juan Ramón Jiménez Hospital of Huelva, Spain from 2018 until 2020.

Blood samples were obtained by venipuncture of the antecubital region using BD Vacutainer SST II tubes with gel separator and advanced vacuum system. Then, the serum was cooled and protected from light for retracting the clot (t = 30 min). After centrifugation (2000 *g*, 10 min) serum samples were frozen at − 80 °C and stored. The study was performed following the principles contained in the Declaration of Helsinki and approved by the Ethics Committee of the regional Andalusian Government (Ethical code num. 1898-N-21).

Patient data were anonymized in a database with a hierarchical control to guarantee secure information access. Clinical parameters such as age, gender, LC or COPD stage, smoking habit, and comorbidities are shown in Table 1.

3. Heteroatom-tagged proteomics for selenoproteins quantification in human serum

The standardless absolute quantification of the main selenoproteins in human serum was carried out by a previously developed analytical method [28] based on two-dimensional separation, combining affinity (AFC) and size exclusion chromatography (SEC), followed by a triple Quad ICP-MS (Agilent Technologies, Tokyo, Japan) using the operational conditions described in Table S1 of the Supplementary Information. Selenium is a heteroatom of the selenoproteins that can act as a "tag" for the selective and sensitive quantification of the selenoproteins. The absolute quantification was performed by isotopic dilution analysis (IDA) using ^{74}Se as the isotopic dilution standard. Thus, an aliquot of 100 μL of human serum was directly injected into a model 1260 Infinity high-performance liquid chromatograph (Agilent Technologies, Tokyo, Japan) equipped with a six-way valve with two 5 mL HiTrap SEC and two affinity columns, heparin-sepharose (HEP-HP) and blue-sepharose (BLU-HP) (GE Healthcare, Uppsala, Sweden). Ammonium acetate was used as mobile phases A (0.05 M, pH = 7.4) and B (1.5 M, pH = 7.4) at a flow rate of 1.3 mL min^{-1}.

Validation of the methodology was carried out with the BCR-637 reference material Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) certified for total selenium content. The precision and accuracy of the analytical method (Table S2) and limits of detection and quantification (Table S3) are detailed in Supplementary Material.

Table 1
Clinical Characteristics of patients included in the study. Healthy Control (HC), Lung cancer (LC), Chronic Obstructive Pulmonary Disease (COPD), COPD patients who developed LC during the follow-up (COPD-LC), very severe COPD (COPD-vs), severe COPD (COPD-s), moderate COPD (COPD-mo) and mild COPD (COPD-mi); DM = Diabetes Mellitus; AH: Arterial Hypertension; DLP: Dyslipidemia; COPD: Chronic Obstructive Pulmonary Disease.

Clinical characteristics		HC (n = 54)	LC (n = 29)	COPD (n = 55)	COPD-LC (n = 16)
Gender	Male	38	20	45	15
	Female	16	9	10	1
Age (years ± SD)		61 ± 11	65 ± 11	63 ± 8	56 ± 8
Smoking habits	Non-smoker	24	7	0	4
	Smoker	15	15	23	4
	Ex-smoker	15	7	28	8
NSCLC Type	Adenocarcinoma	–	18	–	4
	Carcinoid	–	2	–	6
	Squamous cell lung cancer	–	5	–	2
	Epidermoid	–	4	–	4
COPD grading	Mild	–	–	21	4
	Moderate	–	–	9	8
	Severe	–	–	9	0
	Very Severe	–	–	16	4
Comorbidities	DM	5	5	7	3
	AH	17	15	25	11
	DLP	20	18	19	7

3.1. Statistics

Statistica 8 (Statsoft, Tulsa, UK, USA) was used to analyze the data. As Leveńs test revealed no homogenous variance and skewed distribution (checked by normal probability plots), all the data were analyzed by nonparametric methods. Group comparisons were performed using the Krustal-Wallis test and only *p-values* < 0.05 were considered statistically significant. Fold changes (FC) were calculated for any pairwise comparison possible among the studied group by dividing the mean concentrations of selenoproteins and selenometabolites determined in the human serum. The areas under the receiver operating characteristic curve (AUC values from ROC) were also calculated to delve into the specificity and sensitivity of the novel potential biomarkers. In addition, Partial Least Squares–Discriminant Analysis (PLS-DA) and heatmaps were performed to investigate the potential contribution of the selenoproteins and selenometabolites present in human serum to discriminate the studied groups. MetaboAnalyst version 5.0 was used as a statistical tool for the ROC curves and heatmaps (https://www.metaboanalyst.ca/). Spearman correlation analysis was used to determine associations between metals and selenoproteins in the studied groups.

4. Results

4.1. Human serum selenoproteome of LC and COPD with varying severity

The typical selenoproteome profile of human serum is shown in Fig. 1A. Most of the selenium present in serum is in the form of SELENOP, followed by eGPx, SeAlb, and very low concentrations of selenometabolites (SMT). This tendency did not differ between the studied groups, but the average concentrations of those proteins were significantly different among them. The percentage of SeAlb was significantly higher in the LC group (20 %) compared to HC (16 %) (p = 0.002), and COPD (16 %) (p = 0.01), but no significant differences were found in COPD-LC (17 %). The amount of SELENOP was lower (63 %) in the LC group compared to COPD (66 %), COPD-LC (68 %), and HC (67 %), although this difference was only significant (p = 0.006) between the amount of HC and LC groups. eGPx was more significantly abundant in COPD (14 %) when compared to LC (13 %), (p = 0.03), and COPD-LC (10 %), (p = 0.03), respectively. The amount of selenoproteins for COPD subgroups was described in Fig. S1 of the Supplementary Material. Fig. 1C shows the box plot of the averaged selenoproteins and selenometabolites concentrations in HC, LC, COPD, and COPD-LC groups. Similarly, Fig. S2 of the Supplementary Material reports the boxplot corresponding to the concentrations of selenoproteins and selenometabolites in the COPD subgroups (COPD-mi, COPD-mo, COPD-s, and COPD-vs). P-values and AUC values for the selenoproteins and selenometabolites determined in the samples are reported in Table S4 (Supplementary Material). Thus, the concentration of eGPx was significantly greater in the serum of COPD patients when compared to HC (FC = 1.20; $p = 0.003$), as well as COPD patients with LC (FC = 1.47; $p = 0.001$). SELENOP was significantly decreased in LC patients compared

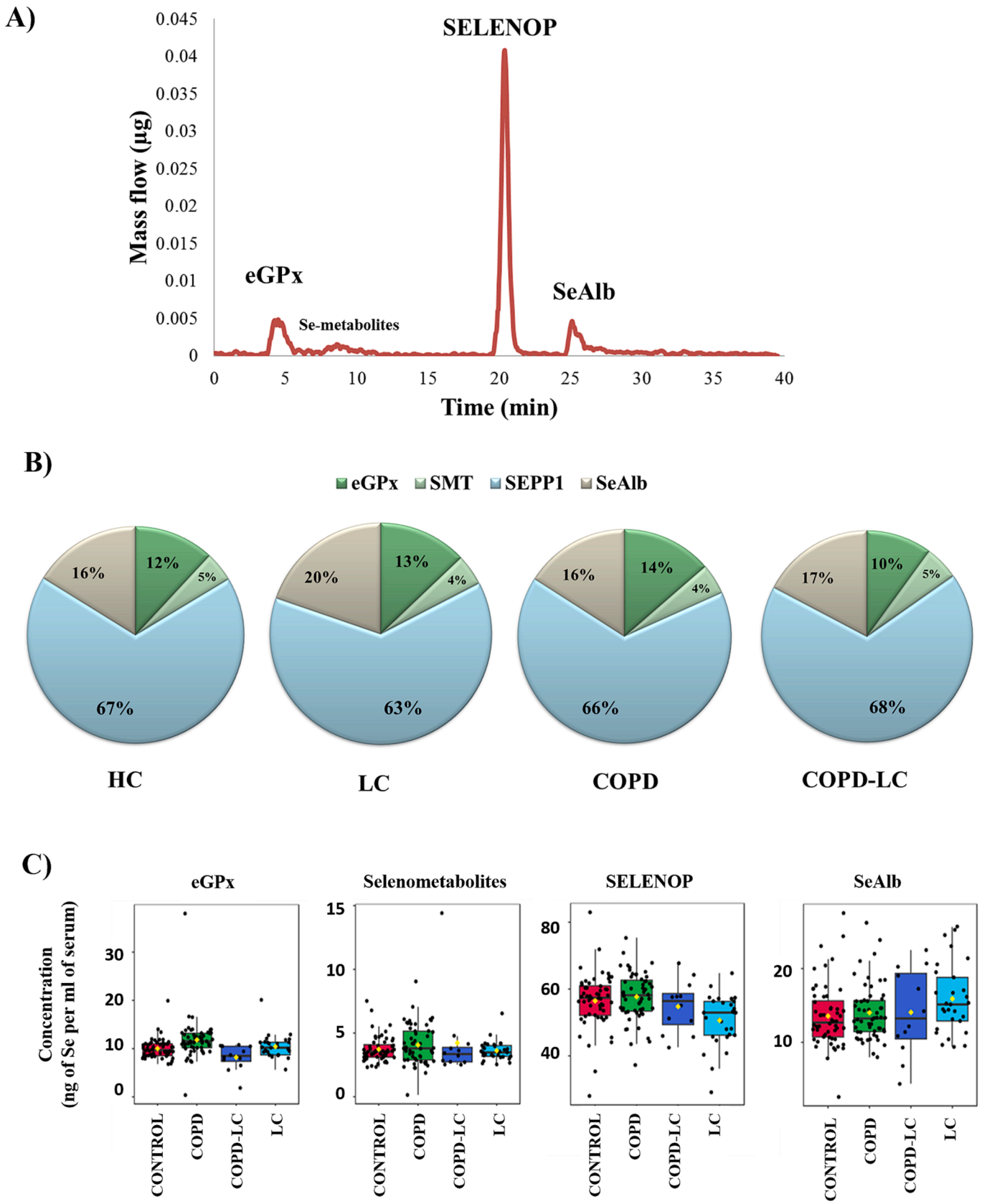

Fig. 1. A) Mass flow chromatogram showing the typical selenoproteome profile of human serum: eGPx (extracellular glutathione peroxidase), SELENOP (selenoprotein P), and SeAlb (selenoalbumin). **B)** Sector diagrams showing the proportion of selenoproteins and selenometabolites in the different groups: HC (control subjects), LC (Lung Cancer patients), COPD (Chronic Obstructive Pulmonary Disease patients), and COPD-LC (COPD patients with LC). **C)** Box plot showing the average concentration (ng of Se per ml of serum sample) in selenoproteins and selenometabolites in human serum samples in HC, LC, COPD, and COPD-LC.

to HC (FC = 0.90; $p = 0.01$) and COPD (FC = 0.88; $p = 0.004$). The concentration of eGPx decreased significantly in mild COPD when compared to healthy controls (FC = 0.82; $p = 0.002$) and increased when compared to COPD patients with LC (FC = 1.59; $p = 0.003$). SELENOP concentrations were lower in the LC group when compared to mild and moderate COPD (FC = 1.15; $p = 0.001$ and FC = 1.18; $p = 0.01$, respectively). No significant differences were found in the concentrations of SeAlb or selenometabolites between the studied groups. Fig. 2 shows a model map summarizing the most important changes in the selenoproteins of the HC, LC, COPD and COPD-LC groups.

Finally, only the AUC value of eGPx in the ROC comparing the COPD and COPD-LC groups showed promise as a potential biomarker with a combined high sensitivity and specificity (AUC = 0.84) (Fig. S3, Supplementary Material).

4.2. Associations between selenoproteins in LC and COPD with varying severity

Spearman correlation analysis was applied to the dataset to determine potential associations between selenoproteins in the different studied groups. After pairwise compassions between the levels of all selenoproteins and total selenometabolites in human serum in the different studied groups, some of them were negatively (rho < 0) or positively (rho > 0) associated, and thus, the concentration of one selenoprotein/total selenometabolites increased as much as the other decreased or *vice versa*, respectively. In addition, in some cases, those significant associations were lineal (rho $\approx \pm 1$). Significant ($p < 0.05$) and strong (rho > 0.5) (Table S5) correlations were calculated and reported in the Supplementary Material. We observed a significant inverse correlation between SELENOP and SeAlb (Fig. 3) in HC (rho = -0.51, $p < 0.05$), LC (rho = -0.76, $p < 0.05$), and COPD-LC (rho = -0.93, $p < 0.05$). This association was stronger in the LC groups than in the control. Moreover, we found that eGPx and selenometabolites were positively correlated in LC patients. No significant associations were observed between selenoproteins and selenometabolites in the COPD group.

5. Discussion

Using the heteroatom-tagged proteomics method of IDA-SEC-AF-ICP-QQQ-MS, we were able to determine the standardless absolute quantification of the main selenoproteins in human serum (~90 % of total selenium). This allowed us to examine the alteration of these metalloproteins in patients with LC and COPD. As far as we know, this is the first study to examine the entire serum selenoproteome (eGPx, SELENOP, and SeAlb) in COPD and COPD-LC patients, as previous studies have only reported concentrations in LC patients using the same methodology [29]. Previous studies have reported the enzymatic activity of the serum antioxidant selenoenzymes eGPx [30] in human serum, as well as the concentration of SELENOP using the molecular technique ELISA [31]. However, few studies specifically explore the identification of selenoproteins as potential biomarkers for LC and COPD diseases.

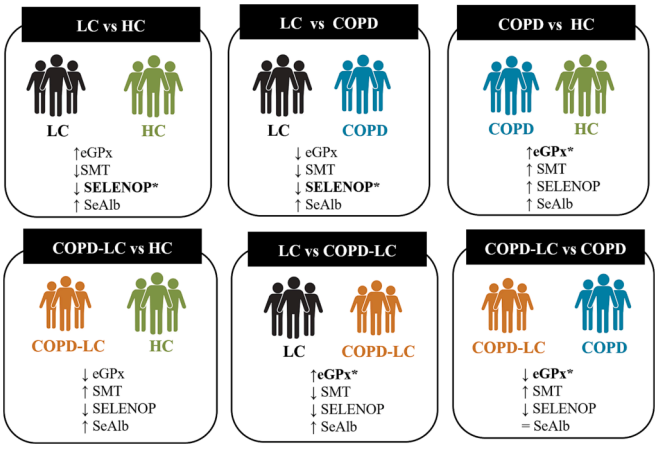

Fig. 2. Model map of the significant dysregulation of the selenoproteins in HC (control subjects), LC (Lung Cancer patients), COPD (Chronic Obstructive Pulmonary Disease patients), and COPD-LC (COPD patients with LC). ↑: elevated concentration (ng Se per ml of serum sample), ↓: diminished concentration (ng Se per ml of serum sample), * Significant value ($p < 0.05$), eGPx (extracellular glutathione peroxidase), SELENOP (selenoprotein P) and SeAlb (selenoalbumin).

We found statistically significant changes in selenoproteins in these tobacco-related diseases, thereby highlighting the importance of the selenoproteome in COPD and LC diseases. We observed a significant reduction in the concentration of SELENOP in LC patients compared to healthy subjects. SELENOP is therefore considered a good biomarker of selenium status [32]. Its main function is the transport of selenium from the liver, where it is mainly synthesized to maintain anti-oxidative selenoenzyme activity in tissues such as the brain and testis [33] and has an inherent antioxidant role since Sec residues act as scavengers of electrons transforming the thiol group into a disulfide bond [34]. These antioxidant properties may ultimately affect lung carcinogenesis [21]. Our results agree with those of Takata et al. [31], who reported that LC patients had slightly lower plasma SELENOP concentrations than healthy controls with borderline statistical significance, and with those of Epplein et al. [21], who reported associations of low levels of SELENOP with an increased risk of LC. In addition to the changes mentioned above in SELENOP concentrations, we also found increased concentrations of eGPx in the serum of COPD patients compared to HC. The GPx family of enzymes catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides using reduced glutathione (GSH) as a substrate [30,35]. GPx presents eight different isoforms including extracellular plasma eGPx [35]. A *meta*-analysis of 10 studies by Zinellu et al. reported a significant decrease in the activity of the selenoprotein GPx-1 in COPD patients compared to individuals without airflow obstruction. However, they found no significant differences in eGPx activity and therefore concluded that the studies on serum samples showed conflicting results [36]. Sotgia et al., [30] have reported reduced GSH concentrations in COPD patients, which has been attributed to a greater consumption of GSH as a co-substrate for the formation of GPx. On the other hand, the significant decrease in the concentration of eGPx only in the COPD-mi group compared to HC may suggest increased levels of oxidative stress especially in mild airflow obstruction. Some authors agree that the evaluation of altered GPx activity may be useful in detecting impairments in the antioxidant defense system in COPD patients [36].

In addition to SELENOP and eGPx, selenium can be present in human serum in the form of selenometabolites, mainly inorganic selenium and selenomethionine, which in turn are associated with albumin (SeAlb) for transport [37]. Interestingly, we found an inverse correlation between SELENOP and SeAlb in HC. The association was strongest in LC and especially, in COPD patients with LC. This association has been described in other types of cancer such as colorectal tumors but not in LC patients [38]. The main function of SeAlb is the transport of selenium. SeAlb levels are increased in the liver following exposure to toxic elements like mercury, which in turn leads to increased production of SELENOP [39]. Some authors have suggested that an increase in SeAlb is indicative of a suppression of the host's immune reaction to the tumor [38]. Thus, our results could suggest that LC patients consumed SELENOP, because its concentrations were found significantly decreased in this group, and SeAlb is being transported to the liver for SELENOP synthesis [40]. This finding could reinforce the hypothesis that SELENOP plays a critical role in LC postulated by other authors [21,31,41], but also the pivotal role of SeAlb only reported in a previous study with few LC human serum samples [24]. We also found a positive correlation between eGPx and selenometabolites in serum from LC patients that

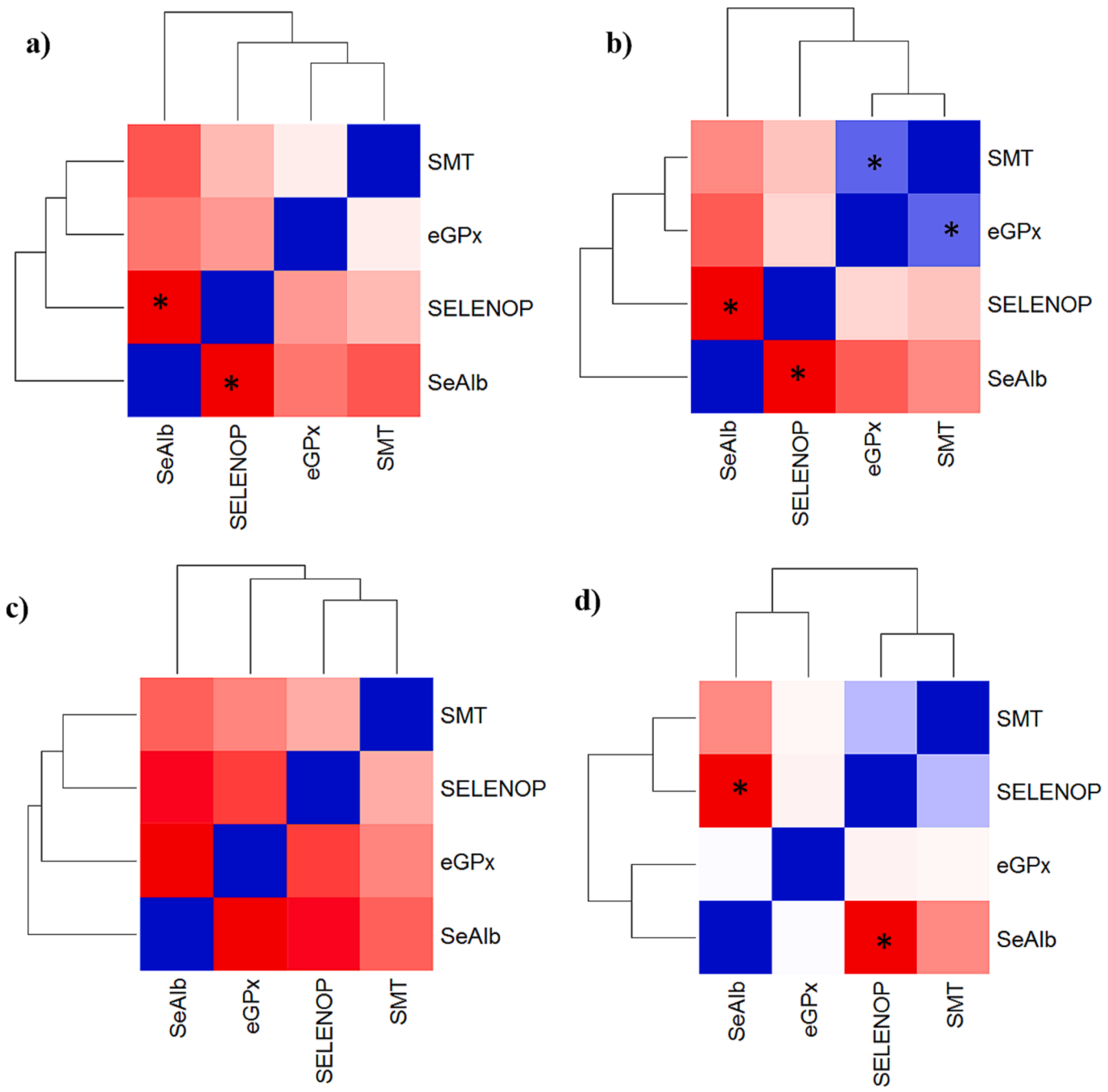

Fig. 3. Spearman Correlation Heatmap between selenoproteins in a) HC, b) LC, c) COPD, and d) COPD-LC groups. *Significant ($p < 0.05$) and strong correlations (rho > 0.5). Red color: negative association, blue color: positive correlation. SMT: Selenometabolites, eGPx: Extracellular glutathione peroxidase, SELENOP: Selenoprotein P, SeAlb: Selenoalbumin.

could be related to an increase in the production of this antioxidant enzyme in response to the oxidative stress posed by cancer. The absence of any association between selenoproteins and selenometabolites in the COPD group was remarkable, with only eGPx being significantly increased in this group.

Many studies have investigated the relationship between LC and COPD, suggesting that chronic airflow obstruction increases the risk of developing LC [42,43], but to the best of our knowledge, selenoproteins have not been previously investigated in this context. We observed significantly increased levels of eGPx and SELENOP in undifferentiated COPD patients when compared to COPD patients with LC and COPD severity subgroups. The decrease of these two proteins in the groups with LC compared to COPD could suggest greater oxidative stress in cancer patients. Some authors have attributed decreased selenoprotein activity to an acceleration of mRNA degradation [44], or nutritional deficiency, including selenium deficiency in this type of patients [45].

Finally, selenoproteins may have potential utility as biomarkers in tobacco-related diseases such as COPD and LC, which are characterized by a significant increase in oxidative stress. In this regard, AUC values greater than 0.75 have been generally considered useful in clinical diagnosis [46]. Our findings may shed some light on the role of selenoproteins as potential biomarkers in LC, COPD, or perhaps LC in the context of pre-existing COPD. In our study, eGPx shows some promise in differentiating patients with COPD and LC from patients with airflow obstruction alone. However, due to the possible limitations in sample size, especially in the COPD-LC group, these results should be validated

in a larger population.

In conclusion, we have determined selenoprotein concentrations representing almost the entire selenoproteome (~90 % of total selenium) in serum samples from LC and COPD patients using a heteroatom-tagged proteomics approach based on IDA-SEC-AF-ICP-QQQ-MS. We found significant differences in some selenoproteins in the different groups studied, such as SELENOP in LC and eGPx in COPD, which could point to critical roles of both selenoproteins in health and disease and might indicate an impaired antioxidant defense system in these tobacco-related diseases. Alterations in the concentrations of these selenoproteins were also found in COPD-LC patients and mild severity of airflow obstruction. Furthermore, the association between SeAlb and SELENOP in LC groups also merits further study. Our data provides novel insights into the role of the selenoproteome in COPD and lung cancer, which may have implications for diagnosis, therapy, and targeted supplementation.

Author contributions

BCL and SSE contributed equally to this work. TGB and GPB are senior authors and equal contributors. The authors' responsibilities were as follows—TGB, BCL and GPB designed the research (project conception and development of the overall research plan). GPB, JLLC, LS: responsible for clinical study, design, and recruitment. CGR, RS, IDO, JMMT, CCM, BGC, AF, ISG, JPT, NFC, CCL, CAD, ARP, LAPF, EMM, MMR, EBV, ALC, CMG, JBGI, CLB, APV: recruitment and collection of the biological samples; BCL and SSE: analyzed serum samples; BCL: visualization; TGB and BCL: supervised the study and assisted in the interpretation of the data; TGB and BCL: drafted the first draft; TGB: funding acquisition and project administration; and all authors have revised and approved the final manuscript.

CRediT authorship contribution statement

Belén Callejón-Leblic: Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Saida Sánchez Espirilla:** Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing. **Carolina Gotera-Rivera:** Resources, Writing – review & editing. **Rafael Santana:** Resources, Writing – review & editing. **Isabel Díaz-Olivares:** Resources, Writing – review & editing. **José María Marín Trigo:** Resources, Writing – review & editing. **Ciro Casanova Macario:** Resources, Writing – review & editing. **Borja G. Cosio:** Resources, Writing – review & editing. **Antonia Fuster:** Conceptualization, Resources, Writing – review & editing. **Ingrid Solanes García:** Resources, Writing – review & editing. **Juan P. de-Torres:** Resources, Writing – review & editing. **Nuria Feu Collado:** Resources, Writing – review & editing. **Carlos Cabrera Lopez:** Resources, Writing – review & editing. **Carlos Amado Diago:** Resources, Writing – review & editing. **Amparo Romero Plaza:** Resources, Writing – review & editing. **Luis Alejandro Padrón Fraysse:** Conceptualization, Resources, Writing – review & editing. **Eduardo Márquez Martín:** Resources, Writing – review & editing. **Margarit Marín Royo:** Resources, Writing – review & editing. **Eva Balcells Vilarnau:** Resources, Writing – review & editing. **Antonia Llunell Casanovas:** Conceptualization, Resources, Writing – review & editing. **Cristina Martínez González:** Resources, Writing – review & editing. **Juan Bautista Galdíz Iturri:** Resources, Writing – review & editing. **Celia Lacárcel Bautista:** Resources, Writing – review & editing. **José Luis Gómez-Ariza:** Conceptualization, Resources, Investigation, Writing – review & editing. **Antonio Pereira-Vega:** Conceptualization, Resources, Investigation, Writing – review & editing. **Luis Seijo:** Conceptualization, Resources, Writing – review & editing. **José Luis López-Campos:** Conceptualization, Resources, Investigation, Writing – review & editing. **Germán Peces-Barba:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing, Resources, Funding acquisition, Project administration. **Tamara García-Barrera:** Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing, Resources, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank all the patients who have volunteered and donated their biomaterials for the study.

Funding

This work has been supported by the project "Heteroatom-tagged proteomics and metabolomics to study lung cancer. Influence of gut microbiota" (Ref.: PY20_00366). Project of Excellence. Regional Ministry of Economy, Knowledge, Business and University, Andalusia, Spain. The authors also thank the grants Ref. 651/2018 and 115/2020 from the Spanish Society of Pneumology and Surgery (SEPAR) and 08/2018 from the Association of Pneumology and Thoracic Surgery (Neumosur) that supported samples recruitment at the hospitals and biobank registration. The authors also thank Instituto de Salud Carlos III (AES16/01783) and unrestricted funding from Menarini Group and AstraZeneca". Funding for open access charge: Universidad de Huelva / CBUA

Ethics approval and consent to participate.

The study was performed in accordance to the principles contained in the Declaration of Helsinki and approved by the Ethical Committee of Andalusian Government (Ethical code num. 1898-N-21). The data of the patients are anonymized in a database with hierarchical access control to guarantee secure information access.

Consent for publication

Consent for publication has been obtained from all patients.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2024.110033.

References

[1] A. Sanz-Medel, "Heteroatom-tagged" quantification of proteins via ICP-MS, Anal. Bioanal. Chem. 408 (2016) 5393–5395, https://doi.org/10.1007/s00216-016-9687-5.
[2] K.E. Hill, R.S. Lloyd, J.G. Yang, R. Read, R.F. Burk, The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame, J. Biol. Chem. 266 (1991) 10050–10053, https://doi.org/10.1016/S0021-9258(18)99185-4.
[3] M.L. Wong, J.F. Medrano, Real-time PCR for mRNA quantitation, Biotechniques 39 (2005) 75–85, https://doi.org/10.2144/05391RV01.
[4] J.L. Bock, The New Era of Automated Immunoassay, 2000. https://academic.oup.com/ajcp/article/113/5/628/1757705.
[5] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, CA Cancer J. Clin. 72 (2022) (2022) 7–33, https://doi.org/10.3322/caac.21708.
[6] B. Callejón-Leblic, A. Arias-Borrego, A. Pereira-Vega, J.L. Gómez-Ariza, T. García-Barrera, The Metallome of Lung Cancer and its Potential Use as Biomarker, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20030778.
[7] G.Y.L. Lui, Z. Kovacevic, V. Richardson, A.M. Merlot, D.S. Kalinowski, D. R. Richardson, Targeting cancer by binding iron: Dissecting cellular signaling pathways, Oncotarget 6 (2015) 18748–18779, https://doi.org/10.18632/oncotarget.4349.

[8] D.J.R. Lane, T.M. Mills, N.H. Shafie, A.M. Merlot, R. Saleh Moussa, D. S. Kalinowski, Z. Kovacevic, D.R. Richardson, Expanding horizons in iron chelation and the treatment of cancer: role of iron in the regulation of ER stress and the epithelial-mesenchymal transition, Biochim. Biophys. Acta 1845 (2014) 166–181, https://doi.org/10.1016/j.bbcan.2014.01.005.
[9] N. Déliot, B. Constantin, Plasma membrane calcium channels in cancer: Alterations and consequences for cell proliferation and migration, BBA 1848 (2015) 2512–2522, https://doi.org/10.1016/j.bbamem.2015.06.009.
[10] L. Fouani, S.V. Menezes, M. Paulson, D.R. Richardson, Z. Kovacevic, Metals and metastasis: Exploiting the role of metals in cancer metastasis to develop novel anti-metastatic agents, Pharmacol. Res. 115 (2017) 275–287, https://doi.org/10.1016/j.phrs.2016.12.001.
[11] V. Thakur, B. Bedogni, The membrane tethered matrix metalloproteinase MT1-MMP at the forefront of melanoma cell invasion and metastasis, Pharmacol. Res. 111 (2016) 17–22, https://doi.org/10.1016/j.phrs.2016.05.019.
[12] J.Y. Kim, H. Bin Lim, M.H. Moon, Online Miniaturized Asymmetrical Flow Field-Flow Fractionation and Inductively Coupled Plasma Mass Spectrometry for Metalloprotein Analysis of Plasma from Patients with Lung Cancer, Anal. Chem. 88 (2016) 10198–10205, https://doi.org/10.1021/acs.analchem.6b02775.
[13] Q. Yang, R.-W. Zhang, P.-C. Sui, H.-T. He, L. Ding, Dysregulation of non-coding RNAs in gastric cancer, World J. Gastroenterol. 21 (2015) 10956–10981, https://doi.org/10.3748/wjg.v21.i39.10956.
[14] P. Sarita, G.J.N. Raju, M.R. Kumar, A.S. Pradeep, S.B. Reddy, Analysis of blood serum of lung cancer patients using particle induced X-ray emission, J. Radioanal. Nucl. Chem. 297 (2013) 431–436, https://doi.org/10.1007/s10967-012-2398-2.
[15] B. Callejón-Leblic, J.L. Gómez-Ariza, A. Pereira-Vega, T. García-Barrera, Metal dyshomeostasis based biomarkers of lung cancer using human biofluids, Metallomics 10 (2018) 1444–1451, https://doi.org/10.1039/C8MT00139A.
[16] G.F. Combs Jr, L.C. Clark, B.W. Turnbull, An analysis of cancer prevention by selenium, Biofactors 14 (2001) 153–159, https://doi.org/10.1002/biof.5520140120.
[17] M.K. Baum, M.J. Miguez-Burbano, A. Campa, G. Shor-Posner, Selenium and Interleukins in Persons Infected with Human Immunodeficiency Virus Type 1, J Infect Dis 182 (2000) S69–S73, https://doi.org/10.1086/315911.
[18] D.G. Menter, A.L. Sabichi, S.M. Lippman, Selenium effects on prostate cell growth, Cancer Epidemiol. Biomark. Prev. 9 (2000) 1171–1182.
[19] T. García-Barrera, J.L. Gómez-Ariza, M. González-Fernández, F. Moreno, M. A. García-Sevillano, V. Gómez-Jacinto, Biological responses related to agonistic, antagonistic and synergistic interactions of chemical species, Anal. Bioanal. Chem. 403 (2012) 2237–2253, https://doi.org/10.1007/s00216-012-5776-2.
[20] L.C. Clark, G.F.J. Combs, B.W. Turnbull, E.H. Slate, D.K. Chalker, J. Chow, L. S. Davis, R.A. Glover, G.F. Graham, E.G. Gross, A. Krongrad, J.L.J. Lesher, H. K. Park, B.B.J. Sanders, C.L. Smith, J.R. Taylor, Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group, J. Am. Med. Assoc. 276 (1996) 1957–1963.
[21] M. Epplein, R.F. Burk, Q. Cai, M.K. Hargreaves, W.J. Blot, A prospective study of plasma selenoprotein P and lung cancer risk among low-income adults, Cancer Epidemiol. Biomark. Prev. 23 (2014) 1238–1244, https://doi.org/10.1158/1055-9965.EPI-13-1308.
[22] V. Mostert, Selenoprotein P: properties, functions, and regulation, Arch. Biochem. Biophys. 376 (2000) 433–438, https://doi.org/10.1006/abbi.2000.1735.
[23] M.E. del C. Busto, C. Oster, S. Cuello-Nuñez, C.L. Deitrich, A. Raab, A. Konopka, W. D. Lehmann, H. Goenaga-Infante, P. Fisicaro, Accurate quantification of selenoproteins in human plasma/serum by isotope dilution ICP-MS: focus on selenoprotein P, J. Anal. At. Spectrom 31 (2016) 1904–1912, https://doi.org/10.1039/C6JA00122J.
[24] B. Callejón-Leblic, G. Rodríguez-Moro, A. Arias-Borrego, A. Pereira-Vega, J. L. Gómez-Ariza, T. García-Barrera, Absolute quantification of selenoproteins and selenometabolites in lung cancer human serum by column switching coupled to triple quadrupole inductively coupled plasma mass spectrometry, J. Chromatogr. A 1619 (2020) 460919, https://doi.org/10.1016/j.chroma.2020.460919.
[25] S. Keith, D.B. Moffett, Z. Rosemond, D.W. Wohlers, R.J. Amata, G.L. Diamond, S. G. Swarts, Toxicological profile for Tungsten, Agency for Toxic Substances and Disease Registry (2005) 153.
[26] P. Bhattacharya, A.H. Welch, K.G. Stollenwerk, M.J. McLaughlin, J. Bundschuh, G. Panaullah, Arsenic in the environment: Biology and Chemistry, Sci. Total Environ. 379 (2007) 109–120, https://doi.org/10.1016/j.scitotenv.2007.02.037.
[27] J. Lamarche, L. Ronga, J. Szpunar, R. Lobinski, Characterization and Quantification of Selenoprotein P: Challenges to Mass Spectrometry, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22126283.
[28] M.A. García-Sevillano, T. García-Barrera, J.L. Gómez-Ariza, Development of a new column switching method for simultaneous speciation of selenometabolites and selenoproteins in human serum, J. Chromatogr. A 1318 (2013) 171–179, https://doi.org/10.1016/j.chroma.2013.10.012.
[29] T. Callejón-Leblic, B. Rodríguez-Moro, G. Arias-Borrego, A. Pereira-Vega, A. Gómez-Ariza, J.L. García-Barrera, Absolute quantification of selenoproteins and selenometabolites in lung cancer human serum by column switching coupled to triple quadrupole inductively coupled plasma mass spectrometry, J. Chromatogr. A 1619 (2020) 460919.
[30] S. Sotgia, P. Paliogiannis, E. Sotgiu, S. Mellino, E. Zinellu, A.G. Fois, P. Pirina, C. Carru, A.A. Mangoni, A. Zinellu, Systematic Review and Meta-Analysis of the Blood Glutathione Redox State in Chronic Obstructive Pulmonary Disease, Antioxidants (Basel) 9 (2020). https://doi.org/10.3390/antiox9111146.
[31] Y. Takata, Y.-B. Xiang, R.F. Burk, H. Li, K.E. Hill, H. Cai, J. Gao, W. Zheng, X.-O. Shu, Q. Cai, Plasma selenoprotein P concentration and lung cancer risk: results from a case–control study nested within the Shanghai Men's Health Study, Carcinogenesis 39 (2018) 1352–1358, https://doi.org/10.1093/carcin/bgy103.
[32] K. Schwarz, C.M. Foltz, Selenium as an integral part of factor 3 against dietary necrotic liver degeneration, J. Am. Chem. Soc. 79 (1957) 3292–3293, https://doi.org/10.1021/ja01569a087.
[33] Y. Saito, Selenoprotein P as an in vivo redox regulator: disorders related to its deficiency and excess, J. Clin. Biochem. Nutr. 66 (2020) 1–7, https://doi.org/10.3164/jcbn.19-31.
[34] F. Li, P.B. Lutz, Y. Pepelyayeva, E.S.J. Arnér, C.A. Bayse, S. Rozovsky, Redox active motifs in selenoproteins, PNAS 111 (2014) 6976–6981, https://doi.org/10.1073/pnas.1319022111.
[35] R. Brigelius-Flohé, M. Maiorino, Glutathione peroxidases, BBA 1830 (2013) 3289–3303, https://doi.org/10.1016/j.bbagen.2012.11.020.
[36] E. Zinellu, A. Zinellu, M.C. Pau, B. Piras, A.G. Fois, S. Mellino, C. Carru, A. A. Mangoni, P. Pirina, Glutathione Peroxidase in Stable Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-analysis, Antioxidants 10 (2021), https://doi.org/10.3390/antiox10111745.
[37] Y. Saito, Selenium Transport Mechanism via Selenoprotein P—Its Physiological Role and Related Diseases, Front. Nutr. 8 (2021), https://doi.org/10.3389/fnut.2021.685517.
[38] M. Roman, P. Jitaru, M. Agostini, G. Cozzi, S. Pucciarelli, D. Nitti, C. Bedin, C. Barbante, Serum seleno-proteins status for colorectal cancer screening explored by data mining techniques - a multidisciplinary pilot study, Microchem. J. 105 (2012) 124–132, https://doi.org/10.1016/j.microc.2012.02.004.
[39] M.A. García-Sevillano, G. Rodríguez-Moro, T. García-Barrera, F. Navarro, J. L. Gómez-Ariza, Biological interactions between mercury and selenium in distribution and detoxification processes in mice under controlled exposure. Effects on Selenoprotein, Chem. Biol. Interact. 229 (2015) 82–90, https://doi.org/10.1016/j.cbi.2015.02.001.
[40] Y. Shiobara, K.T. Suzuki, Binding of selenium (administered as selenite) to albumin after efflux from red blood cells, J. Chromatogr. BBiomed Sci Appl 710 (1998) 49–56, https://doi.org/10.1016/s0378-4347(98)00142-x.
[41] P. Gresner, J. Gromadzinska, E. Jablonska, J. Kaczmarski, W. Wasowicz, Expression of selenoprotein-coding genes SEPP1, SEP15 and hGPX1 in non-small cell lung cancer, Lung Cancer 65 (2009) 34–40, https://doi.org/10.1016/j.lungcan.2008.10.023.
[42] B.A. Parris, H.E. O'Farrell, K.M. Fong, I.A. Yang, Chronic obstructive pulmonary disease (COPD) and lung cancer: Common pathways for pathogenesis, J. Thorac. Dis. 11 (2019) S2155–S2172, https://doi.org/10.21037/jtd.2019.10.54.
[43] A.L. Durham, I.M. Adcock, The relationship between COPD and lung cancer, Lung Cancer 90 (2015) 121–127, https://doi.org/10.1016/j.lungcan.2015.08.017.
[44] A.J. Dabo, W. Ezegbunam, A.E. Wyman, J. Moon, C. Railwah, A. Lora, S.M. Majka, P. Geraghty, R.F. Foronjy, Targeting c-Src Reverses Accelerated GPX-1 mRNA Decay in Chronic Obstructive Pulmonary Disease Airway Epithelial Cells, Am. J. Respir. Cell Mol. Biol. 62 (2020) 598–607, https://doi.org/10.1165/rcmb.2019-0177OC.
[45] H. Fritz, D. Kennedy, D. Fergusson, R. Fernandes, K. Cooley, A. Seely, S. Sagar, R. Wong, D. Seely, Selenium and lung cancer: a systematic review and meta analysis, PLoS One 6 (2011) e26259.
[46] J. Fan, S. Upadhye, A. Worster, Understanding receiver operating characteristic (ROC) curves, CJEM 8 (2006) 19–20, https://doi.org/10.1017/s1481803500013336.