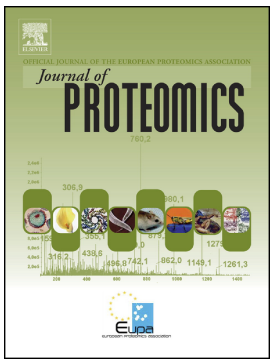

Alterations in plasma concentrations of energy-balance-related metabolites in patients with lung, or head & neck, cancers: Effects of radiotherapy

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Alterations in plasma concentrations of energy-balance-related metabolites in patients with lung, or head & neck, cancers: Effects of radiotherapy

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Running title: Energy metabolism in lung, or head & neck, cancers

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ABSTRACT

We investigated the alterations in the plasma concentrations of energy-balance-related metabolites in patients with lung (LC) or head & neck (HNC) cancer and the changes on these parameters induced by radiotherapy. The study was conducted in 33 patients with non-small cell LC and 28 patients with HNC. We analyzed the concentrations of 17 metabolites involved in glycolysis, citric acid cycle and amino acid metabolism using targeted gas chromatography coupled to quadrupole time-of-flight mass spectrometry. For comparison, a control group of 50 healthy individuals was included in the present study. Patients with LC or HNC had significant alterations in the plasma levels of several energy-balance-related metabolites. Radiotherapy partially normalized these alterations in patients with LC, but not in those with HNC. The measurement of plasma glutamate concentration was an excellent predictor of the presence of LC or HNC, with sensitivity >90% and specificity >80%. Also, associations with disease prognosis were observed with plasma glutamate, amino acids and β-hydroxybutyrate concentrations. *Significance:* These results extend the knowledge of metabolic alterations in cancer, thus facilitating the search for biomarkers and therapeutic targets.

Keywords: cancer metabolism; glutamate; β-hydroxybutyrate; metabolomics; radiotherapy

Abbreviations: Atg7, autophagy related 7 gene; AUC, area under the curve; BCAA, branched chain amino acids; HNC, head & neck cancer; LC, lung cancer; PLSDA, partial least squares discriminant analysis; ROC, receiver operating characteristics; RT, radiotherapy; VIP, variable importance in projection.

1. Introduction

Currently, lung cancer (LC) and head & neck cancer (HNC) are two of the most common malignant diseases, and are among the main causes of cancer death worldwide [1,2]. Both types of cancer occur in epithelium-coated organs that are exposed to multiple environmental insults. Genetic susceptibility, tobacco smoking habit, and toxic agents contribute to the etiology of these types of cancer. The current treatment of LC and HNC is the combination of surgery, radiotherapy (RT) and chemotherapy. Although RT is an effective treatment, its response is very variable; reflecting the heterogeneity of the disease and its sensitivity to treatment [3]. Recently, targeted therapies in selected sub-populations of patients and the advances in immunotherapy have achieved remarkable improvements in clinical outcomes [4]. However, in locally-advanced disease only a small minority of these patients will achieve long-term survival [5]. Hence, a deeper knowledge of the biology of the disease is needed to unravel effective therapeutic strategies.

One of the characteristics of cancer is a deregulation of cellular energy metabolism which encourages tumor cell growth and proliferation [6]. Cancer cells undergo glycolysis even in aerobic conditions (Warburg effect) [7] and show a remarkable metabolic flexibility, including alterations in lipid mitochondrial and amino acid metabolism [8]. The development of metabolomics methods has provided sensitive and effective tools to investigate the relationships between metabolism and cancer and, in particular, the modification of energy metabolism by tumors to acquire increased amounts of ATP for their increased growth and uncontrolled development. Understanding the features and complexity of the cancer energy metabolism will help

develop new approaches in identification of early diagnostic and prognostic biomarkers, which are the current targets for effective cancer therapy.

This study was aimed at investigating alterations in plasma concentrations of energy-balance-related metabolites in patients with LC and HNC, together with the changes produced by RT on these parameters with a view to an insight into the association between these alterations and the clinical condition of the patients.

2. Patients and methods

2.1. Study design and patient population

The study was performed in 33 patients with non-small cell LC [26 male (79%); mean age = 72 (range 65-79) years] and 28 patients with HNC [25 male (89%); mean age = 65 (range 56-73) years] needing radical RT. All patients attended the Department of Radiation Oncology of our hospital. All patients had a Karnofsky Index > 70 and were classified as 0 or 1 on the Eastern Cooperative Oncology Group scale [9]. The radiation schedule was normofractionated RT (total dose 60-66 Gy at 2 Gy/day, 5 days/week for LC, and total dose 60-70 Gy at 2 Gy/day, 5 days/week for HNC, depending on disease stage), using the Volumetric Modulated Arc Therapy method [10] (Varian RapidArc®, Varian Medical Systems, Palo Alto, CA, USA). Acute toxicity assessment during RT was performed weekly using the criteria of the Radiation Therapy Oncology Group and the European Organization for Research and Treatment of Cancer [11]. Fourteen LC patients were also treated with cisplatin (50 mg/m^2) and etoposide (50 mg/m^2) IV every 3 weeks. Eleven HNC patients received cisplatin (100 mg/m^2) IV every 3 weeks concomitant with radiation therapy. Prior to irradiation, and one month post-RT, blood samples were obtained and EDTA-plasma aliquots were stored at -80ºC until processed

for metabolomics analyses. A detailed description of the clinical characteristics of the LC and HNC patients are shown in Table 1.

Fifty healthy subjects were included as control group [25 male (50%); mean age = 42 (range 35-47) years]. These subjects were participants in a population-based study carried out in our geographical area. They had no analytical or clinical evidence of neoplasia, infectious disease, hepatic damage, renal insufficiency, or mental illness [12]. All patients and control subjects signed a written informed consent according to the declaration of Helsinki. The study was approved by the Ethics Committee (Institutional Review Board) of the *Hospital Universitari de Sant Joan* (project code: 14/2017).

2.2 Metabolomic analysis of plasma samples

We analyzed the concentrations of 17 metabolites involved in glycolysis, citric acid cycle and amino acid metabolism, as we have described previously [13]. We employed a 7890A gas chromatograph coupled to a 7200 quadrupole time-of-flight mass spectrometer with an electron impact source, and equipped with a 7693 autosampler module and a J&W Scientific HP-5MS column (J&W Scientific HP-5MS column, 30 m × 0.25 mm, 0.25 µm, Agilent Technologies, Santa Clara, CA, USA).

2.3. Statistical analyses

The Mann-Whitney *U*-test was used to evaluate differences between any two groups of quantitative variables. The χ-square test was employed to assess differences in qualitative variables. Binary logistic regression analysis was used to investigate independent associations between clinical and demographic characteristics, metabolite variations, and the presence or absence of disease. Receiver operating characteristics (ROC) curve analysis was employed to evaluate the diagnostic accuracy

of the measured metabolites. This analysis employs plots of different sensitivity/specificity pairs based on varying decision thresholds. Sensitivity (or true positive rate) is the proportion of the sample correctly identified as associating with a specific disease. Specificity (or true negative rate) is the proportion of subjects correctly identified as not segregating with a specific disease. False positive rate is calculated as 1-specificity. The area under the curve (AUC) represents the ability of the test to correctly classify patients with the alteration being investigated. The values of AUC can range between 1 (perfect test) and 0.5 (worthless test) [14]. Multivariate statistics were used to improve the analysis of complex raw data and pattern recognition. Linear discriminant analysis was employed as a method of classification and principal component analysis as an unsupervised data analysis method to segregate the compared groups according to metabolomic data. We also performed a multivariate analysis of pattern recognition, including the supervised partial least squares discriminant analysis (PLSDA). The relative magnitude of observed changes was evaluated using the variable importance in projection (VIP) score [15]. All statistical analyses and relevant graphics were performed with GraphPad Prism software 6.01 (GraphPad Software, San Diego, CA, USA), SPSS Software (IBM SPSS Statistics for Windows, Version 25.00 Armonk, NY: IBM Corp.) and MetaboAnalyst 3.0 (www.metaboanalyst.ca). Differences were considered statistically significant when the p value was < 0.05.

3. Results

3.1. Alterations in energy balance-related metabolites in patients with LC or HNC

Pre-RT, patients with LC had major alterations in plasma concentrations of metabolites involved in glycolysis, citric acid cycle and amino acid metabolism,

compared to the control group. We found significant decreases in plasma β-hydroxybutyrate, isoleucine, leucine, serine, and valine, and increases in aspartate, glutamate, malate, pyruvate, and succinate concentrations. These alterations were partially reversed following RT. The treatment tended to normalize the plasma concentrations of β-hydroxybutyrate, aspartate, isoleucine, leucine, and serine. However, RT was associated with further increases in the concentrations of aconitate, fumarate, and malate (Fig. 1 and Supplementary Table 1).

Pre-RT, patients with HNC had significantly decreased plasma leucine, serine, and valine, and increased glutamate, malate, pyruvate, and succinate concentrations. In general (and contrary to LC), RT did not normalize these alterations and, in addition, patients had significant decreases in plasma concentrations of β-hydroxybutyrate and valine, and increased concentrations of aconitate, glutamate, glutamine, pyruvate and succinate (Fig. 2 and Supplementary Table 2).

3.2. Identification of the most relevant metabolites

The score plot of the PLSDA analysis showed that the control group and the LC patients pre-RT were two different populations with little overlap, and that RT produced changes in metabolite concentrations that tended towards the control group values (Fig. 3A). To identify the metabolites that showed the most relevant alterations pre-RT, we calculated the VIP scores. This score is a measure of the variable's degree-of-alteration associated with the disease i.e. a higher VIP score is considered more relevant in disease status classification. The VIP analysis identified glutamate and serine as the metabolites presenting the most relevant pre-RT alterations, while glutamate and pyruvate were the most relevant metabolites post-RT (Fig. 3B). ROC plots showed that the measurement of pre-RT plasma glutamate concentration was an

excellent predictor of the presence of LC, with an AUC > 0.90; higher than that of pre-RT serine and post-RT glutamate and pyruvate (Fig. 3C and D). The analytical sensitivity was 97% and specificity was 82% at glutamate = 3 μM. Binary logistic regression analysis showed that the pre-RT differences between LC patients and controls with respect to plasma concentrations of β-hydroxybutyrate, glutamate, isoleucine, leucine, serine, and valine were maintained when adjusted for age, gender and other confounding factors (Supplementary Table 3).

As in patients with LC, the score plot of the PLSDA analysis showed that the control group and the HNC patients pre-RT were clearly two distinct populations. However, in these patients, RT did not produce any appreciable trend towards normalization of the metabolite values (Fig. 4A). The VIP analysis identified glutamate and serine as the most relevant pre-RT metabolites, while glutamate and glutamine were the most relevant metabolites post-RT (Fig. 4B). ROC plots showed the measurement of post-RT plasma glutamate concentration as an excellent predictor of the presence of HNC, with an AUC close to unity, and superior to that of pre-RT glutamate and serine and of post-RT glutamine (Fig. 4C and D). The analytical sensitivity was 100% and specificity was 92% at glutamate = 3.37 μM. Binary logistic regression analysis showed that only the pre-RT differences between HNC patients and controls in the plasma values of glutamate and serine were maintained when adjusted for age, gender and other confounding factors (Supplementary Table 4).

3.3. Relationships between the measured metabolites and the clinical characteristics of the patients

In patients with LC, the presence of metastasis was associated with higher pre-RT β-hydroxybutyrate concentrations. Local tumor recurrence three years post-

diagnosis was associated with higher pre-RT leucine, valine and fumarate concentrations. Higher post-RT glutamate concentrations were associated with a higher incidence of *exitus*. In patients with HNC, we found higher pre-RT plasma glutamate concentrations in patients with local tumor recurrence, while higher serine, lactate and α-ketoglutarate values were associated with *exitus* at three years post-diagnosis. According to the analysis of the ROC curves, these parameters showed high sensitivities and low specificities in discriminating patients with respect to the selected clinical characteristic. Specifically, in LC patients, pre-RT leucine and valine showed a sensitivity of 0.92 in detecting the appearance of local tumor recurrence, β-hydroxybutyrate showed a sensitivity of 0.80 in detecting appearance of metastases, and post-RT glutamate showed a sensitivity of 0.90 in detecting *exitus* in the short-to-medium term. In HNC patients, pre-RT glutamine had a sensitivity of 0.89 in detecting local tumor recurrence, and serine had a sensitivity of 0.89 in detecting *exitus* in the short-to-medium term (Fig. 5 and Supplementary Table 5). We did not find any significant differences in any of the most relevant metabolites in relation to other clinical characteristics (data not shown), nor did we find differences based on whether patients received concomitant chemotherapy, or not (Supplementary Tables 6 and 7).

4. Discussion

Glutamate is a key amino acid in metabolic pathways. The first step in glutaminolysis is the conversion of glutamine to glutamate, a process catalyzed by glutaminase in the mitochondria [16]. Glutamate is an anaplerotic substrate for the citric acid cycle. It contributes to the carbon backbone, particularly in conditions of carbon diversion to glycolytic pathways [17]. Transfer of an amino group from glutamate to oxaloacetate by aspartate aminotransferase results in α-ketoglutarate

and aspartate, while nitrogen transfer from glutamate to pyruvate by alanine aminotransferase results in α-ketoglutarate and alanine. By means of these reactions, glutamate activates multiple biochemical pathways including protein and nucleic acid synthesis, epigenetic modifications, interchange of metabolites between the mitochondria and the cytosol, and stimulation of the antioxidant defense systems [18-21]. In the present study, the glutamate increase in patients with LC or HNC was accompanied by increased plasma concentrations of aspartate, malate, pyruvate, and succinate; reflecting an activation of citric acid cycle. Indeed, several lines of evidence suggest that LC evidences what has been termed "glutamine addiction" i.e. continued functioning of the citric acid cycle which is necessary for the growth and proliferation of tumor cells requires the anaplerotic replenishment of intermediates which is achieved, mostly, by activation of glutaminolysis *via* glutamate synthesis [22-26].

Several studies have suggested that glutamate concentrations have a great impact on the tumor's fate. For example, in mouse models of LC, deletion of autophagy related 7 (*Atg7*) gene decreases macroautophagy, increases oxidative stress, suppresses tumor growth, and promotes tumor cell death [27,28]. LC cells need autophagy to buffer metabolic stress induced by the tumor's low-oxygen microenvironment i.e. autophagy promotes degradation of intracellular components needed for the *de novo* synthesis of amino acids, nucleotides, fatty acids and sugars [29]. However, while *Atg7* deficiency decreases citric acid cycle intermediates such as glutamate, aspartate, and α-ketoglutarate [27], supplementation with glutamine or glutamate causes a compensatory adaptation that increases LC cell survival by inducing glutathione synthesis and augmenting the capacity of the antioxidant defense system of the tumor cells [30]. Also, NADPH oxidase 4, an enzyme that is abundantly

expressed in LC, promotes glutaminolysis, increases glutamate and glutathione concentrations, and contributes to LC cell survival [22].

The consequences of high glutamate concentration in patients with LC or HNC (and its role in tumor resistance) may be important in treatment response. Recent reports indicate that glutamine/glutamate-dependent upregulation of intracellular glutathione concentrations is associated with radiation resistance in cancer [31]. Indeed, exposure of several lines of cancer cells to anoxia/re-oxygenation stress induced upregulation of the glutaminolysis pathway, and altered glutamine utilization have been associated with a decrease in cellular oxidative stress, increase in glutathione concentrations, and improved tumor cell survival, even including exposure to ionizing radiation [32,33]. In the present study, RT was associated with normalization of the plasma concentrations of serine and branched-chain amino acids as well as with a further increase in some citric acid cycle intermediates (fumarate and malate) in patients with LC. Glutamate concentrations remained unchanged. RT did not ameliorate alterations of metabolites in patients with HNC but, instead, was associated with an increase in glutamate and citric acid cycle intermediates such as malate, pyruvate and succinate. The long-term clinical consequences of these effects (or lack thereof) need to be explored systematically.

Recently, the identification of non-invasive biomarkers to improve the diagnosis, prognosis and evaluation of the response to treatment in patients with various types of cancer has gained considerable attention. Of note in the current study were the very remarkable alterations in plasma glutamate concentrations in patients with LC or with HNC. The statistical analyses using ROC curves showed that measuring this amino acid has a high diagnostic accuracy in differentiating between patients with

these cancers, and healthy individuals i.e. highlights its possible usefulness as a biomarker. These results agree with several studies showing that the measurement of plasma glutamate concentration has a high sensitivity and specificity in differentiating between LC and benign lung lesions [34]. Likewise, a high plasma glutamate concentration associates with low survival [35] and the presence of neurological complications in patients with LC [36]. On the other hand, glutamate and other glutaminolysis-related products can act as biomarkers of chemotherapy efficacy in patients with oral squamous cell carcinoma [37,38]. The measurement of plasma concentrations of glutamate by metabolomics, alone or in combination with other parameters, has demonstrated its usefulness as a biomarker in patients with pancreatic cancer [39-42].

Lactate: pyruvate molar ratio reflects the equilibrium between the product and the substrate of the reaction catalyzed by lactase dehydrogenase, and indirectly reflects the NADH: NAD+ cytoplasmic redox state [43,44]. In the present study, we obtained a micromolar ratio between lactate and pyruvate of 9.6 in healthy individuals (Supplementary Tables 1 and 2), which is consistent with the expected values under physiological conditions. This ratio decreased in patients with LC (pre-RT: 7.6 and post-RT: 7.0) as well as in patients with HNC (pre-RT: 7.3 and post-RT: 5.3). These changes may be the result of increased levels of NAD+ over NADH suggesting a shift of the redox equilibrium towards oxidation. The increase in oxidation status has also been seen in other tumor processes as a result of increased oxidative stress both at the tissue and systemic levels [45].

Also of note in the present study is the important decrease in circulating concentrations of serine, leucine and isoleucine pre-RT. This could be interpreted as an

enhanced cellular demand for these metabolites related to increased glutaminolysis. The high demand for glutamine leads to an increased uptake by the tumor cells, of serine and branched chain amino acids (BCAA) including leucine and isoleucine. These amino acids provide an important source for the biosynthesis of glutamine and glutamate. The increased demand on glutamine would explain the reduced serum concentrations of serine and BCAA in actively replicating tumor cells [46-48].

Other relationships between energy balance-related metabolites and the clinical fate of our patients were noted. The small number of patients in the study indicates that these findings should be interpreted with caution. However, the results do suggest further studies to be undertaken to investigate the possible utility of these metabolites as prognostic biomarkers. For example, patients with LC and metastases had higher plasma concentrations of β-hydroxybutyrate, while those with local tumor recurrence had higher values of leucine, valine and fumarate. There is a dearth of Information on the relationships between these metabolites and the prognosis of cancer. β-hydroxybutyrate acts as a signaling molecule [49] that increases the expression of forkhead box O and mammalian target of rapamycin and, as such, stimulates cell growth, proliferation and longevity [50,51]. β-hydroxybutyrate also increases the antioxidant capacity of cells by inducing the synthesis of metallothioneins, superoxide dismutase and catalase [52]. In cancer, β-hydroxybutyrate administration accelerates the rate of mammary tumor growth in mice [53] and in cultured breast cancer cells [54], while the plasma concentrations of this metabolite are increased in patients with thyroid cancer [55]. Our results also showed that patients with LC or HNC having local tumor recurrence or *exitus* had higher plasma concentrations of BCAA, serine, and some metabolites associated with

glutaminolysis (glutamine, α-ketoglutarate and fumarate). These results could indicate a poorer prognosis is associated with deregulation of glutaminolysis.

In conclusion, the present study shows that patients with LC or HNC have significant alterations in the plasma concentrations of several energy balance-related metabolites, and that RT partially normalizes these alterations in patients with LC; but not in those patients with HNC. In addition, the study identifies glutamate as the most severely altered metabolite. We also report associations between plasma glutamate, BCAA and β-hydroxybutyrate concentrations and disease prognosis. Knowledge of the metabolic alterations in cancer encourages the search for biomarkers and therapeutic targets. A caveat of the present study is the low number of patients investigated. Further studies with more casuistry, and conducted in different centers, are necessary to confirm these hypotheses

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Meritxell Arenas and Jordi Camps designed the initial study; Mònica Arguís, Meritxell Arenas, Mauricio Murcia, Sebastià Sabater and Laura Torres provided study samples

and associated data; Elisabet Rodríguez-Tomàs, Salvador Fernández-Arroyo, Gerard Baiges-Gayà and Anna Hernández-Aguilera carried out the measurements and analysed the data; Meritxell Arenas, Jordi Camps and Jorge Joven wrote the manuscript.

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FIGURE LEGENDS

Fig. 1 Alterations in energy balance-related metabolites in patients with lung cancer (LC) pre-radiotherapy (RT) and post-RT compared with the control group (A and B, respectively). The left panels show the heatmaps and the right panels are schematic presentation of the implicated metabolic pathways. Significantly increased metabolites ($p < 0.05$) are shown in red and decreased metabolites are shown in blue.

Fig. 2 Alterations in energy balance-related metabolites in patients with head & neck cancer (HNC) pre-radiotherapy (RT) and post-RT compared with the control group (A and B, respectively). The left panels show the heatmaps and the right panels are schematic presentation of the implicated metabolic pathways. Significantly increased metabolites ($p < 0.05$) are shown in red and decreased metabolites are shown in blue.

Fig. 3 (A) Partial least squares discriminant analysis (PLSDA). Plots of plasma samples from lung cancer (LC) patients pre- and post-radiotherapy (RT) and the control group. (B) Variable importance in projection (VIP) scores of the PLSDA in patients with LC. The most relevant metabolite changes were for glutamate and serine pre-RT, and for glutamate and pyruvate post-RT. (C) Receiver operating characteristics plots of plasma glutamate and serine concentrations in LC patients pre-RT, relative to the control group. (D) Receiver operating characteristics plots of plasma glutamate and pyruvate concentrations in LC patients post-RT, relative to the control group. AUC = area under the curve. [a] $p < 0.05$; [b] $p < 0.01$.

Fig. 4 (A) Partial least squares discriminant analysis (PLSDA). Plots of plasma samples from head & neck (HNC) patients and the control group pre- and post-radiotherapy (RT). (B) Variable importance in projection (VIP) scores of the PLSDA in patients with HNC. The most relevant metabolite changes were for glutamate and serine pre-RT, and for glutamate and pyruvate post-RT. (C) Receiver operating characteristics plots of plasma glutamate and serine concentrations in HNC patients pre-RT, relative to the control group. (D) Receiver operating characteristics plots of plasma glutamate and pyruvate concentrations in HNC patients post-RT, relative to the control group. AUC = area under the curve. [a] $p < 0.05$; [b] $p < 0.01$.

Fig. 5 Plasma concentrations of selected metabolites and receiver operating characteristics curves in relation to the clinical characteristics of patients with lung cancer (A) and with head & neck cancer (B). AUC: Area under the curve. RT: Radiotherapy. [a] $p < 0.05$.

This study analyzed the changes produced in the plasma concentrations of energy-balance-related metabolites in patients with lung cancer or head and neck cancer. The results obtained identified glutamate as the parameter with the highest discrimination capacity between patients and the control group. The relationships between various metabolites and clinical outcomes were also analyzed. These results extend the knowledge of metabolic alterations in cancer, thus facilitating the search for biomarkers and therapeutic targets.

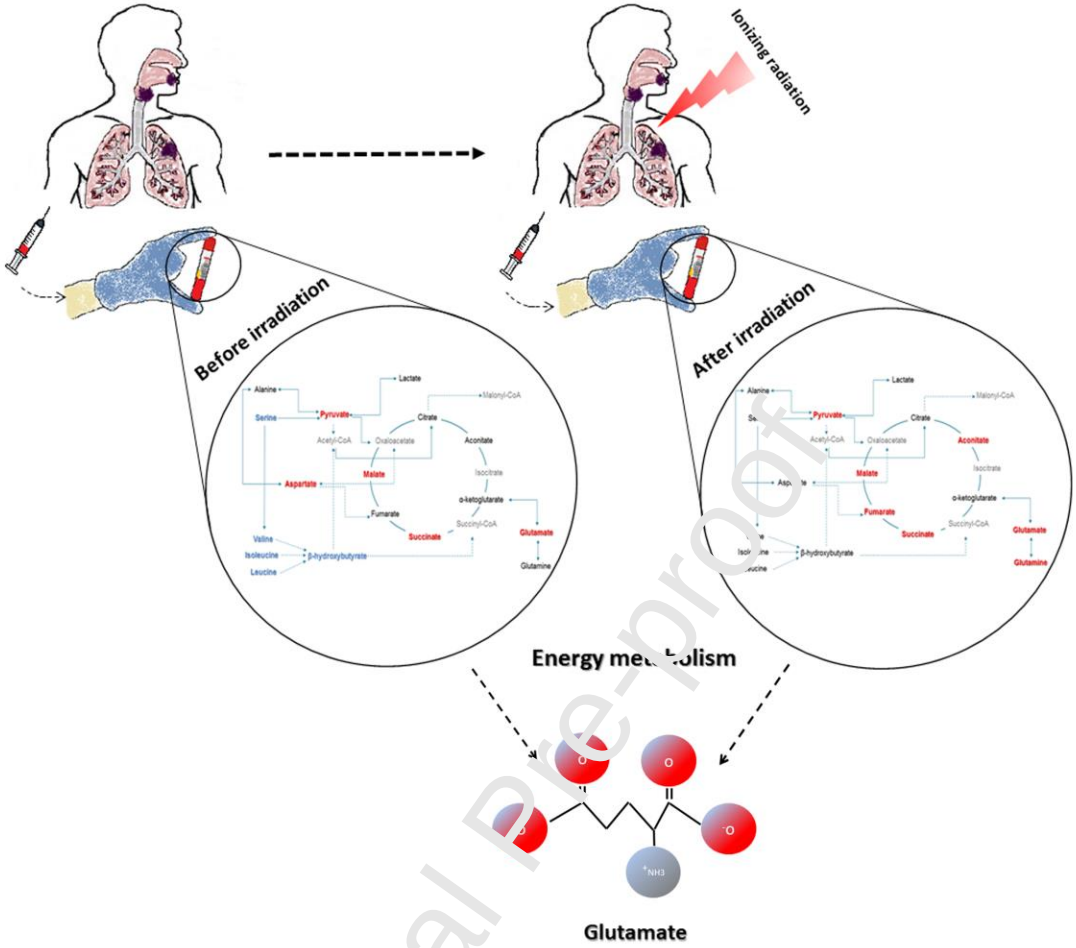
Table 1

Clinical characteristics of the lung cancer and the head & neck cancer patients, and the control group

	Control group n = 50	Lung cancer n = 33	Head & Neck cancer n = 28	p-value
Clinical and demographic characteristics				
Age, years	42 (35–47)	72 (65–79)	65 (56–73)	< 0.001
Male sex, n (%)	25 (50.0)	26 (78.8)	25 (89.3)	< 0.001
Alcohol habit (>20g/day), n (%)	15 (30.0)	8 (24.2)	11 (39.3)	0.042
Smoking habit, n (%)	17 (34.0)	14 (42.4)	16 (57.1)	< 0.001
Number of cigarettes/day <10 10-20 >20	 8 (16.0) 9 (18.0) 0 (0.0)	 1 (3.0) 8 (24.2) 5 (15.2)	 0 (0.0) 9 (32.1) 7 (25.0)	 < 0.001
Hypertension, n (%)	4 (8.0)	19 (57.6)	17 (60.7)	< 0.001
Diabetes mellitus, n (%)	5 (10.0)	10 (30.3)	6 (21.4)	0.085
Cancer stages	N.A.			
Stage I		14 (42.4)	5 (17.9)	
Stage II		4 (12.1)	5 (17.9)	
Stage III		15 (45.5)	5 (17.9)	
Stage IV		0 (0.0)	13 (46.4)	
Histology	N.A.			
Squamous carcinoma		18 (54.5)	26 (92.9)	
Adenocarcinoma		13 (39.4)	1 (3.6)	
Others		2 (6.0)	1 (3.6)	
Secondary effects of radiotherapy	N.A.			
Epithelitis Grade 0 Grade 1 Grade 2 Grade 3		 9 (27.3) 18 (54.5) 5 (15.2) 1 (3.0)	 3 (10.7) 10 (35.7) 15 (53.6) 0 (0.0)	
Lung toxicity Grade 0 Grade 1 Grade 2 Grade 3		 8 (24.2) 17 (51.5) 6 (18.2) 2 (6.1)	 N.A.	
Xerostomia Grade 0 Grade 1 Grade 2 Grade 3		 N.A.	 6 (21.4) 8 (28.6) 12 (42.9) 2 (7.1)	
Mucositis		N.A.	20.0 (71.4)	
Esophagitis		N.A.	6.0 (21.5)	
Exitus*	N.A.	19 (57.6)	9 (32.1)	
Metastasis*	N.A.	11 (33.3)	0 (0.0)	
Local tumor recurrence*	N.A.	14 (42.4)	9 (32.1)	
Follow-up (months)	N.A.	40 (13–43)	36 (12–41)	

Values are shown as number of cases and percentages (in parentheses) or medians and interquartile ranges (in parentheses. N.A.: Not applicable. Statistical analyses were

performed with the χ-squared test, except for age, which was with the Mann-Whitney *U*-test. *These characteristics were evaluated three years after the end of the study.



Highlights

- Patients with cancer have alterations in energy-balance metabolism
- Plasma glutamate discriminated patients with lung cancer from healthy people
- Similar results were obtained in patients with head & neck cancer
- Radiotherapy did not importantly ameliorate these alterations
- Several metabolites were associated with the clinical outcomes