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E-mail: jzus_b@zju.edu.cn

Correspondence

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Analysis of volatile organic compounds in exhaled breath after radiotherapy

Dianlong GE[1,2], Xue ZOU[1]✉, Yajing CHU[1,2], Jijuan ZHOU[1,2], Wei XU[1,2], Yue LIU[1], Qiangling ZHANG[1], Yan LU[1,3], Lei XIA[1], Aiyue LI[1], Chaoqun HUANG[1], Pei WANG[3]✉, Chengyin SHEN[1,3], Yannan CHU[1]

[1]*Anhui Province Key Laboratory of Medical Physics and Technology, Institute of Health and Medical Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, China*
[2]*University of Science and Technology of China, Hefei 230026, China*
[3]*Department of Laboratory Medicine, Hefei Cancer Hospital, Chinese Academy of Sciences, Hefei 230031, China*

Radiotherapy uses high-energy X-rays or other particles to destroy cancer cells and medical practitioners have used this approach extensively for cancer treatment (Hachadorian et al., 2020). However, it is accompanied by risks because it seriously harms normal cells while killing cancer cells. The side effects can lower cancer patients' quality of life and are very unpredictable due to individual differences (Bentzen, 2006). Therefore, it is essential to assess a patient's body damage after radiotherapy to formulate an individualized recovery treatment plan. Exhaled volatile organic compounds (VOCs) can be changed by radiotherapy and thus used for medical diagnosis (Vaks et al., 2012). During treatment, high-energy X-rays can induce apoptosis; meanwhile, cell membranes are damaged due to lipid peroxidation, converting unsaturated fatty acids into volatile metabolites (Losada-Barreiro and Bravo-Díaz, 2017). At the same time, radiotherapy oxidizes water, resulting in reactive oxygen species (ROS) that can increase the epithelial permeability of pulmonary alveoli, enabling the respiratory system to exhale volatile metabolites (Davidovich et al., 2013; Popa et al., 2020). These exhaled VOCs can be used to monitor body damage caused by radiotherapy.

✉ Xue ZOU, xzou@cmpt.ac.cn
Pei WANG, 61775618@qq.com
Xue ZOU, https://orcid.org/0000-0001-9540-6764
Pei WANG, https://orcid.org/0000-0002-8613-6480

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In our study, 110 breath samples were obtained from ten lung cancer patients who were receiving radiotherapy. The gender, age, tumor location, cancer stage, and daily dose of the patients are shown in Table 1. We stored the samples in our lab-made glass bottles away from light to avoid interference from sampling bags or tubes. The VOCs in the samples were detected using solid phase micro-extraction-gas chromatography/mass spectrometry (SPME-GC/MS). Ethanol was used as the internal standard. We evaluated the changing trends of exhaled VOCs and identified the radiotherapy biomarkers through multivariate statistical analysis.

Table 1 Demographic characteristics of involved subjects

No.	Gender	Age (years)	Tumor location	Cancer stage
1	Male	72	Upper right lung	T3N3M0 IIIC
2	Female	58	Upper right lung	T2N3M0 IIIB
3	Male	71	Left lung	T1bN3M0 IIIB
4	Male	66	Lower right lung	T2N2M1c IVB
5	Male	65	Right lung	T2N2M1b IVA
6	Male	70	Right lung	T2N2M0 IIIA
7	Male	49	Right lung	T4N3M0 IIIC
8	Male	56	Lower left lung	T4N2M0 IIIB
9	Male	62	Upper left lung	T4N0M0 IIIA
10	Male	72	Upper right lung	T4N3M1c IVB

The daily dose was 2 Gy.

As shown in the heatmap in Fig. 1, 28 kinds of VOCs regularly changed after radiotherapy. The data are arranged horizontally in five columns, representing the concentrations of exhaled VOCs 0.5 h before

and 0.5, 2.0, 4.0, and 6.0 h after radiotherapy. We discovered three change trends which occurred together: (1) increasing in the 6.0 h after treatment, including hexanal, butyric acid, and propionic acid; (2) decreasing in the 6.0 h after treatment, including triacetin, indene, cyclohexanone, methylbenzene, ethylbenzene, 1,3-dimethylbenzene, and 1,4-dimethylbenzene; (3) increasing followed by decreasing, including acetic acid, valeric acid, hexanoic acid, nonanoic acid, acetone, decanal, 2-methylbutanoic acid, 2-ethylhexanal, 2-ethylhexanol, L-menthol, dimethyl adipate, dodecane, tetradecane, caprolactam, *p*-cresol, 6-methylheptan-2-one, 6-methyl-5-hepten-2-one, and indole. However, for propionic acid in (1), the concentration before treatment was higher than that 0.5 h after treatment, which did not entirely follow the first change trend. Meanwhile, for triacetin in (2), the concentration abnormally increased 2.0 h after treatment, which was not entirely in line with the second change trend.

The 28 VOCs could be classified into aromatic compounds, alkanes, aldehydes, acids, ketones, alcohols, and esters. Aromatic compounds, alkanes, and aldehydes are biomarkers of lipid oxidation of polyunsaturated fatty acids in cell membranes (Phillips et al., 2013). The increase of alkanes and aldehydes may be due to lipid oxidation accelerated by radiotherapy or irradiation (Phillips et al., 2013). However, the reason for the decreased concentration of aromatic compounds in this study was unclear. Meanwhile, acids, ketones, alcohols, and esters are products of the reaction between glutathionesulfonic acid and glucose, or between glutathione and glucose. Nakajima (2015) observed that glutathionesulfonic acid and alutathione increased in the liver after radiation exposure to protect the organ against oxidative stress, which was inducible even by low-dose radiation exposure.

We analyzed the breath samples 0.5 h before and after treatment to identify the exhaled biomarkers of radiotherapy. Based on the Mann-Whitney *U* test, seven kinds of VOCs ($P<0.05$) were significantly different: acetone, propionic acid, 2-methylbutanoic acid, indene, dimethyl adipate, triacetin, and indole. We selected these VOCs for the orthogonal partial least squares-discriminant analysis (OPLS-DA) on SIMCA-P14.1. Fig. 2a presents the corresponding integral matrix; the integral values are concentrated in the T2 ellipse of the scatter plot at a 95% confidence interval. The before-and-after breath samples exhibited a promising

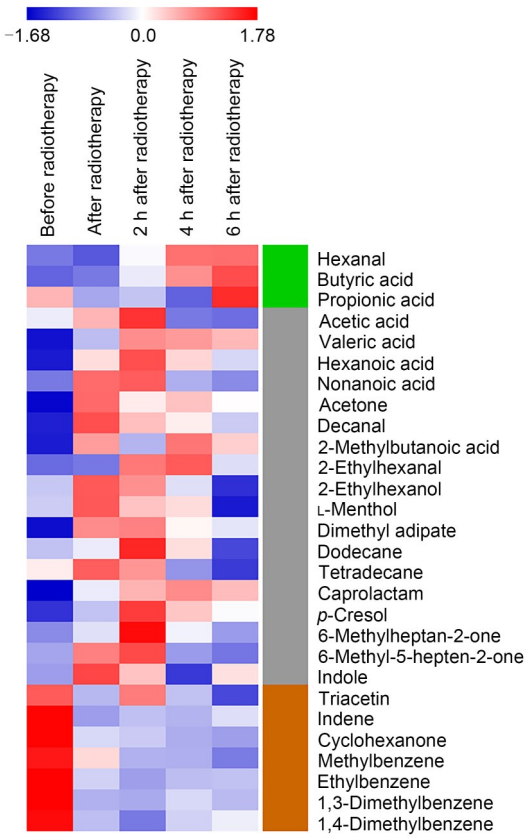

Fig. 1 Heatmap of exhaled volatile organic compounds (VOCs) after radiotherapy. VOC concentrations from high to low are represented by a horizontal color gradient, with blue indicating low concentrations and red indicating high ones. Exhaled VOCs were classified into three groups according to the change trends. As shown in the label bar on the right, green denotes an increasing trend, gray denotes an increasing then decreasing trend, and brown denotes a decreasing trend.

separation trend. R^2X (cum), which we used to evaluate the quality of the OPLS-DA model, was 0.912. On the other hand, R^2Y (cum), which assessed the proportion of variance of the response variable, was 0.631. Meanwhile, the statistical value Q^2 (cum) calculated from sevenfold cross-validation described the model's predictive ability. In this model, Q^2=0.53>0.50, indicating that the original model had a high predictive capability.

The supervised pattern-recognition method tended to generate an overfitting phenomenon while enlarging the differences between the groups. Therefore, it was necessary to apply the permutation test to prove the model's validity. We obtained validation plots from 200 permutation tests. In Fig. 2b, R^2 and Q^2 regression lines intersect the left *Y*-axis. The R^2Y (cum) and Q^2Y (cum) values of the original OPLS-DA model were

more significant than all R^2Y and Q^2Y values of the permutated models, thus validating the original model's suitability and validity. The respective intercepts of R^2Y and Q^2Y were 0.0472 and −0.1900 (both of which were less than 0.4), signifying a non-overfitted model. The goal of determining the variable importance in projection (VIP) scores was to select the VOCs that most significantly contributed to the OPLS-DA model, revealing three determinant VOCs with VIP scores greater than 1.0: propionic acid, triacetin, and indole. We identified these VOCs as the biomarkers of radiotherapy.

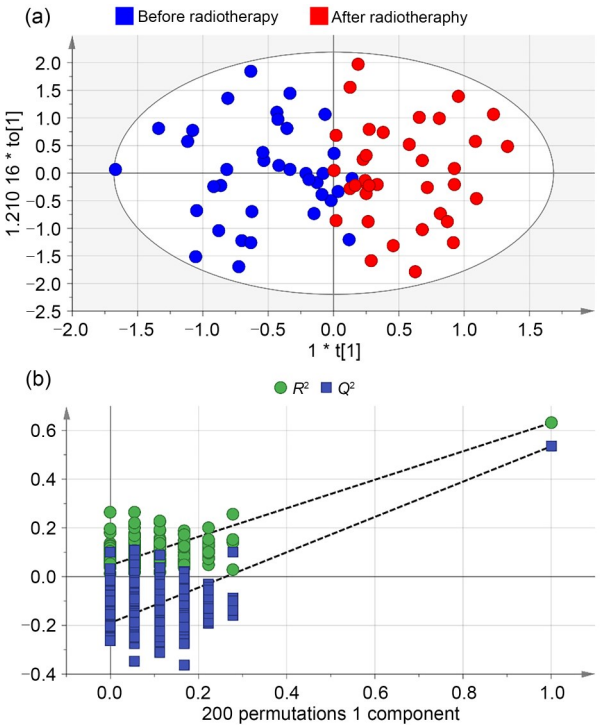

Fig. 2 Establishment of orthogonal partial least squares-discriminant analysis (OPLS-DA) models. (a) OPLS-DA score plot; (b) OPLS-DA validation plot (*Y*-axis represents R^2Y or Q^2Y, *X*-axis represents the correlation between displacement model and original model, and *Y*-axis intercepts: R^2=(0, 0.0472) and Q^2=(0, −0.1900)).

Fig. S1 presents the quantitative analysis results for the three biomarkers. Meanwhile, Fig. 3 illustrates the median concentration values and interquartile ranges (IQRs) of the three characteristic VOCs. The concentrations of propionic acid and triacetin in the exhaled breath of subjects decreased after radiotherapy (Figs. 3a and 3b), while that of indole increased (Fig. 3c).

Exhaled propionic acid is a potential lung cancer biomarker produced from lung cancer cells (Wang et al., 2014). It can also originate from the fermentation of undigested carbohydrates by intestinal anaerobes (Lin, 2004). From the heatmap in Fig. 1, it is evident that its concentration immediately decreased after radiotherapy before gradually increasing. Presumably, the initial decrease was due to lung cancer cell death caused by the treatment, while the subsequent increase was due to the rising number of intestinal anaerobes; intestinal anaerobes multiplied because of the intestinal inflammation caused by radiotherapy (McKelvey et al., 2018).

Nonspecific lipases and esterases can hydrolyze triacetin to release glycerol and acetic acid. In this study, triacetin concentration decreased after treatment. Glycerol can undergo conversion to glyceraldehyde-3-phosphate for glycolysis, while acetic acid can produce acetyl-coenzyme A (acetyl-CoA) (Reisenauer et al., 2011; Long et al., 2015). Radiotherapy can increase the activity of the hypoxia-inducible factor 1 (HIF-1) transcription factor (Zhong et al., 2013), which then stimulates glycolysis and consumes glycerol (Semenza, 2011). Additionally, radiotherapy can cause the overexpression of cytoplasmic acetyl-CoA synthetase in tumors (Chung et al., 2017), which consumes acetic acid. Thus, it can be inferred that glycerol and acetic acid consumption causes a decrease in triacetin concentration.

Indole is related to tryptophan metabolism in intestinal flora (de Vietro et al., 2020). In this study, its concentration immediately increased after radiotherapy before gradually decreasing. Moreover, it can bind to blood albumin (Gambhir et al., 1975). However, the molecular conformation of albumin can change under ionizing radiation (Kirilova et al., 2011). Therefore, we assumed that the initially increased concentration was caused by a decrease in the ability of indole to bind to albumin. In another study, indole produced by intestinal flora decreased when exposed to radiation (Broin et al., 2015). This may be why we found that exhaled indole concentration decreased 2.0 h after radiotherapy.

Notably, the time intervals between the most significant concentration differences for the 28 VOCs before and after radiotherapy were different (Fig. 1). For instance, the time interval for dodecane was 2.0 h, while that for methylbenzene was 6.0 h. This situation raises the question: what is the appropriate time point to sample exhaled gases? We suggest recruiting more

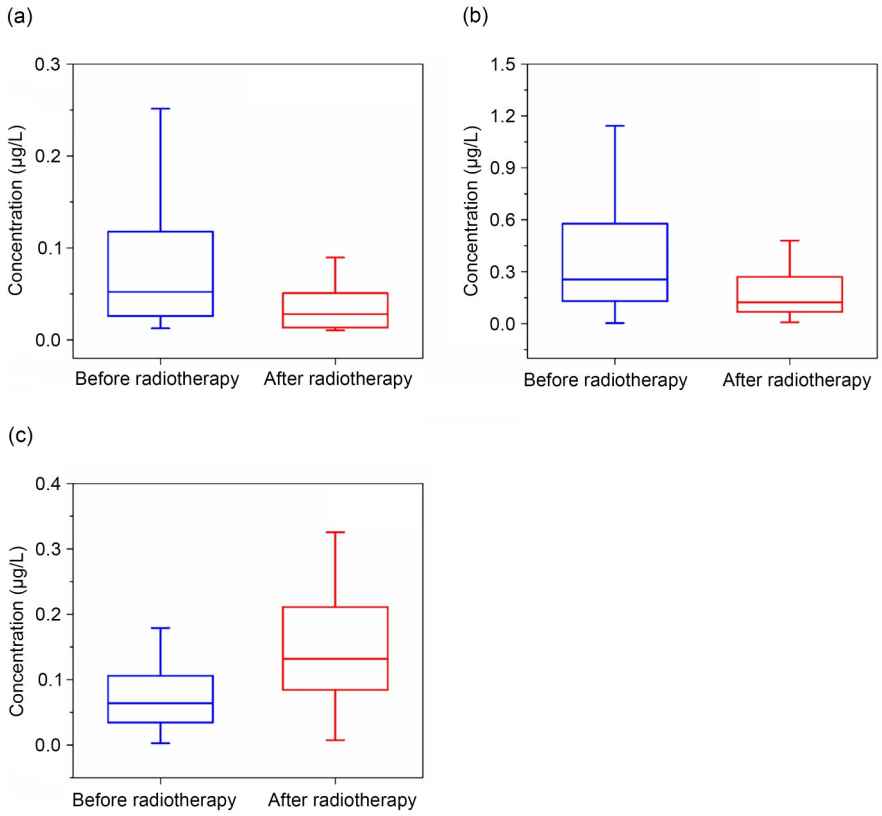

Fig. 3 Box-whisker plots of the medium concentrations and interquartile ranges (IQRs) of propionic acid (a), triacetin (b), and indole (c) in the exhaled breath of subjects before and after radiotherapy.

volunteers and sampling breath more frequently, which we plan to undertake in subsequent studies.

In summary, we found that 28 kinds of VOCs changed regularly after radiotherapy, including aromatic compounds, alkanes, aldehydes, acids, ketones, alcohols, and esters. Using the Mann-Whitney *U* test and OPLS-DA analysis, we identified three radiotherapy biomarkers. Furthermore, we established a discriminant model with a Q^2 value of 0.53, indicating good predictive ability. Based on heatmap analysis, we pinpointed a problem regarding the appropriate time point to sample exhaled gases. The time intervals between the most significant concentration differences before and after radiotherapy for the 28 VOCs were different. This context requires further study involving more breath samples and higher sampling frequency.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Dianlong GE, Pei WANG, Xue ZOU, and Chengyin SHEN contributed to the conception and design of this study. Dianlong GE, Yajing CHU, Jijuan ZHOU, Wei XU, Yue LIU, and Qiangling ZHANG performed the experiments. Dianlong GE, Yan LU, Lei XIA, and Aiyue LI performed the data analysis. Dianlong GE wrote the manuscript. Chaoqun HUANG, Pei WANG, Xue ZOU, Chengyin SHEN, and Yannan CHU

revised the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Dianlong GE, Xue ZOU, Yajing CHU, Jijuan ZHOU, Wei XU, Yue LIU, Qiangling ZHANG, Yan LU, Lei XIA, Aiyue LI, Chaoqun HUANG, Pei WANG, Chengyin SHEN, and Yannan CHU declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all patients for being included in the study.

References

Bentzen SM, 2006. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer*, 6(9):702-713. https://doi.org/10.1038/nrc1950

Broin PÓ, Vaitheesvaran B, Saha S, et al., 2015. Intestinal microbiota-derived metabolomic blood plasma markers for prior radiation injury. *Int J Radiat Oncol Biol Phys*, 91(2):360-367. https://doi.org/10.1016/j.ijrobp.2014.10.023

Chung YH, Tsai CK, Wang CC, et al., 2017. Early response monitoring following radiation therapy by using [^{18}F]FDG and [^{11}C]acetate PET in prostate cancer xenograft model with metabolomics corroboration. *Molecules*, 22(11):1946. https://doi.org/10.3390/molecules22111946

Davidovich N, DiPaolo BC, Lawrence GG, et al., 2013. Cyclic stretch-induced oxidative stress increases pulmonary alveolar epithelial permeability. *Am J Respir Cell Mol Biol*, 49(1):156-164. https://doi.org/10.1165/rcmb.2012-0252OC

de Vietro N, Aresta A, Rotelli MT, et al., 2020. Relationship between cancer tissue derived and exhaled volatile organic compound from colorectal cancer patients. Preliminary results. *J Pharm Biomed Anal*, 180:113055. https://doi.org/10.1016/j.jpba.2019.113055

Gambhir KK, McMenamy RH, Watson F, 1975. Positions in human serum albumin which involve the indole binding site. Sequence of 107-residue fragment. *J Biol Chem*, 250(17):6711-6719. https://doi.org/10.1016/S0021-9258(19)40991-5

Hachadorian RL, Bruza P, Jermyn M, et al., 2020. Imaging radiation dose in breast radiotherapy by X-ray CT calibration of Cherenkov light. *Nat Commun*, 11:2298. https://doi.org/10.1038/s41467-020-16031-z

Kirilova EM, Kalnina I, Zvagule T, et al., 2011. Fluorescent study of human blood plasma albumin alterations induced by ionizing radiation. *J Fluoresc*, 21(3):923-927. https://doi.org/10.1007/s10895-010-0608-2

Lin J, 2004. Too much short chain fatty acids cause neonatal necrotizing enterocolitis. *Med Hypotheses*, 62(2):291-293. https://doi.org/10.1016/S0306-9877(03)00333-5

Long PM, Tighe SW, Driscoll HE, et al., 2015. Acetate supplementation as a means of inducing glioblastoma stem-like cell growth arrest. *J Cell Physiol*, 230(8):1929-1943. https://doi.org/10.1002/jcp.24927

Losada-Barreiro S, Bravo-Díaz C, 2017. Free radicals and polyphenols: the redox chemistry of neurodegenerative diseases. *Eur J Med Chem*, 133:379-402. https://doi.org/10.1016/j.ejmech.2017.03.061

McKelvey KJ, Hudson AL, Back M, et al., 2018. Radiation, inflammation and the immune response in cancer. *Mamm Genome*, 29(11):843-865. https://doi.org/10.1007/s00335-018-9777-0

Nakajima T, 2015. Roles of sulfur metabolism and rhodanese in detoxification and anti-oxidative stress functions in the liver: responses to radiation exposure. *Med Sci Monit*, 21:1721-1725. https://doi.org/10.12659/MSM.893234

Phillips M, Byrnes R, Cataneo RN, et al., 2013. Detection of volatile biomarkers of therapeutic radiation in breath. *J Breath Res*, 7(3):036002. https://doi.org/10.1088/1752-7155/7/3/036002

Popa C, Maia Bratu A, Petrus M, 2020. Special issue "applications of photoacoustic spectroscopy". *Molecules*, 25(21):5116. https://doi.org/10.3390/molecules25215116

Reisenauer CJ, Bhatt DP, Mitteness DJ, et al., 2011. Acetate supplementation attenuates lipopolysaccharide-induced neuroinflammation. *J Neurochem*, 117(2):264-274. https://doi.org/10.1111/j.1471-4159.2011.07198.x

Semenza GL, 2011. Oxygen sensing, homeostasis, and disease. *N Engl J Med*, 365(6):537-547. https://doi.org/10.1056/nejmra1011165

Vaks VL, Domracheva EG, Sobakinskaya EA, et al., 2012. Using the methods and facilities of nonsteady-state spectroscopy of the subterahertz and terahertz frequency ranges for noninvasive medical diagnosis. *J Opt Technol*, 79(2):66-69. https://doi.org/10.1364/jot.79.000066

Wang CS, Dong R, Wang XY, et al., 2014. Exhaled volatile organic compounds as lung cancer biomarkers during one-lung ventilation. *Sci Rep*, 4:7312. https://doi.org/10. 1038/srep07312

Zhong J, Rajaram N, Brizel DM, et al., 2013. Radiation induces aerobic glycolysis through reactive oxygen species. *Radiother Oncol*, 106(3):390-396. https://doi.org/10.1016/j.radonc. 2013.02.013

Supplementary information

Materials and methods; Fig. S1