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Rapid Identification of Cancerous Cell Lines and Evaluation of Cytotoxic Effects on Cancer Cells with VUV-PIMS

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Abstract

Distinguishing tumor from normal and exploring candidate drugs for anti-cancer activity serve as two of most main impetuses for development of cancer therapeutics. In this study, a vacuum ultraviolet photoionization mass spectrometer (VUV-PIMS) has been applied for the headspace trace-level characteristic of the volatile organic compounds (VOCs) emitted by in-vitro cultured human cells. To validate VUV-PIMS' potential for cell or tissue diagnostics by cancer-related VOC biomarkers, four species of cancerous and one non-cancerous cell lines were selected, i.e. HeLa, A549, HepG-2, MGC-803 and 293T, derived from cervical carcinoma, lung cancer, hepatoma, gastric cancer and non-malignant control respectively. Furthermore, to clarify whether VUV-PIMS is a proper analytical tool that allow evaluating candidate drug effects against cancerous cell lines, the VOCs profiling of cell lines exposed to 50nM polyphyllin and vitamin C (Vc) were measured as well. As a result, dozens of VOCs were found to be significantly distinctive released between cancerous and non-cancerous cell lines, which can be used as volatile cancer markers. Additionally, dimethyl sulphide (DMS) were substantially more released form cancerous cells than non-cancerous cells after exposure of polyphyllin. Accordingly, we propose a mechanism by which cysteamine is accumulated in cancerous cells and metabolized to DMS on a large scale when confronting with some sort of cytotoxic effects (autophagy and apoptosis). Therefore, this technique is not only reliable enough to recognize cancerous cell lines, but also a supplementary method to explore how drug-related cytotoxic effects will influence the metabolism of cancer cells.

Keywords: *VUV-PIMS, Cytotoxic effects, Cancer cells, Dimethyl sulphide*

1. Introduction

For the several most common malignancies-cancers of cervix, lung, liver and stomach are the most vital risk factors, who have both higher incidence rates and mortality [1]. However, in the process from prognosis to surgery, rapid identification of cancer cells (tissue) and characterize the effects of the cytotoxic chemotherapeutic drugs make both physician and surgeon confused. To simplify the matter, cell lines derived from human tumors have been widely applied as experimental models of neoplastic disease. Due to tumor growth is

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associated with gene and protein expression changes [2], cancer cells create distinctive VOC profiles from normal cells. Cancer VOCs are produced by tumor cells, then they are exchanged and excreted via various body fluids and excreted into the endobronchial cavity [3]. Rely on these VOC profiles in urine [4], blood [5], exhaled breath [6][7][8][9][10][11][12] and tissue [13], a potentially non-invasive frontier in the diagnosis of cancer is heralded. Cytotoxicity level in cancer cells was normally express as IC_{50} value (the dose necessary to cause a 50% reduction in cell viability relative to untreated control cells). Previous researches suggested the cytotoxic effects triggered by several first-line cancer therapeutic drugs and their combination [14][15]. However, to what extend anti-cancer drugs will influence the VOC profiles of cancerous cell lines have never been reported.

A variety of instruments have been used to identify cancer-related VOCs. GC-MS associated with several pre-concentration techniques remains the gold standard hybrid-analytical platform applied successfully for uncovering new biomarkers over a diverse range of diseases [16][17][18]. These methods providing quite integral lists of cancer-related VOCs, but due to not offering real-time measurement, suffering from sample preparation, they are not suitable to measure reactive VOCs and can't be used clinically. Additionally, rapid evaporative ionization mass spectrometry (REIMS) has been reported as an intraoperative tissue identify method [13]. However, it should be collocated with electrosurgical devices and measuring it production of "smoke", which means characterization can't be made unless the electrosurgical knife has cut down. Proton transfer reaction mass spectrometry (PTR-MS) was also carried out for online and offline characterization of VOC biomarkers from cancer cells [19]. Nevertheless, the detected VOC biomarkers were limited to molecules with a proton affinity greater than that of water [20], among which there were not extremely specific cancer-related VOCs observed by the online measurement of PTR-MS.

As another type of instrument, offering online identification of VOCs, VUC-PIMS is known as soft-photoionization and fragment free [21][22]. In this study, our newly developed ultrasensitive VUV-PIMS was used for rapid online discrimination of cancerous cell lines and evaluate cytotoxic effects on cancer cells. Favorable reliability and reproducibility were verified in such approach. Specific peaks at m/z=62 which is considered as DMS were detected in all polyphyllin treated cell lines, especially produced from cancerous cell lines. A divergent pathway of tumor biochemistry under cytotoxic stress was hypothesized, which suggests that DMS concentration might be served as a new index to evaluate the cytotoxic levels of anti-cancer drugs.

2. Materials and methods

2.1. Cell culture

Four human original malignant and one benign cell lines were employed in the study viz., lung adenocarcinoma (A549), cervix (HeLa), liver (HepG-2), gastric carcinoma (MGC-803) and kidney benign cells (293T). These cell lines were stored in Research Center for Eco-Environmental Sciences (Beijing, China). The cells have grown in DMEM culture medium (Gibico) supplemented with 10% fetal bovine serum (Hyclone), 1% penicillin/streptomycin (Corning, 0.25%). All cell lines were cultured in 25cm2 flasks in 10mL culture medium and permitted to grow for a minimum of 48h. .Cells were seeded at densities of 80-100 x $l0^4$cells/mL (more than 90% coverage rate) such that the cells were in plateau phase of growth before they were introduced into the VUV-PIMS measure system or exposed to polyphyllin, vitamin C. Three repetitions were taken in each group and the pure medium controls were

also incubate 48h. For those drug-treated cell lines, the most clinically effective concentration of 50 nM polyphyllin and vitamin C with 24h exposure time were employed [23][24]. For all experiments, cell lines were cultured under 37℃ in humidified atmosphere containing 5% CO_2.

2.2. VOCs measurement system

The measurement system is consisted of VUV-PIMS, 37℃ incubator, 37℃ sterile buffer box and sterile filter et al. [Figure 1]. Cell lines were incubated in the 25cm^2 cultivation flasks which were closed with a Teflon plug. Each Teflon plug was punch with two holes allowing a pair of 1/8 outer diameter steel tubing across. In order to ensure proper mixing of the head-space air, one of the tubing connected with VUV-PIMS sampling tube was protruded 3-5 cm deeper than the other one, which was the inlet of synthetic air (80%N_2, 20% O_2) and CO_2 stream. A 37℃ sterile buffer box was installed between sterile filter and inlet tubing, avoiding the dramatic fluctuation of gas component in the cultivation flask. The buffer box should be filled with synthetic air and CO_2 stream for several minutes before a measurement is conducted. The flux of synthetic air and CO_2 set as 0.2 L/min and 0.01 L/min respectively.

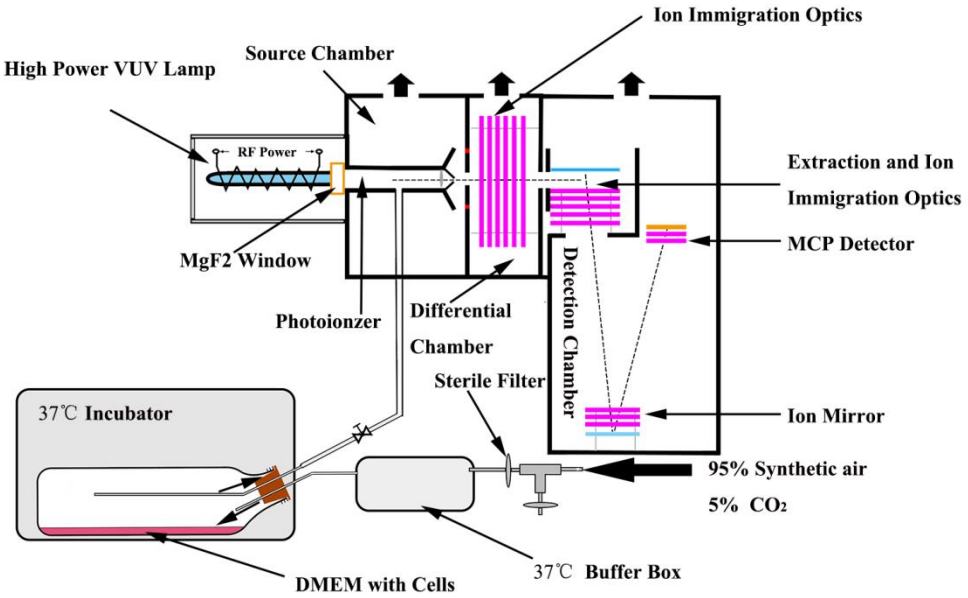

Figure 1. The VOCs measurement system is mainly consisted of VUV-PIMS, 37℃ incubator, 37℃ sterile buffer box and sterile filter. 95% synthetic air and 5% CO2 was introduced into 37℃ sterile buffer box consecutively

The VUV-PIMS was newly developed in our laboratory, which had been previously described elsewhere [25]. Briefly, it was characterized with a VUV photoionzation source, a sample inlet, and a short V-shaped time-of-flight mass spectrometer (TOF-MS). The VUV photoionzation source was based on a krypton lamp outputting a photon flux of ~5 × 10^{14} photon/s at 123.9 nm [26].The ambient sample inlet consists of a stainless steel tube (1/8 inch outer diameter) and a precision needle valve. The sample flow was maintained at ~1 cm^3 s^{-1} without preconcentration. The TOF-MS was equipped with a free flight distance of 460 mm, chevron microchannel plates, a 100× amplifier (Ortec VT120C), and a TOF multiscaler (FAST Comtec, P7888). The pressure of the detection chamber was set as ~3.5 × 10^{-3} Pa.

2.3. Reagents and gases

In order to evaluate how the VOC metabolism will be influenced by drug treatment, polyphyllin (BBI, 99%) or Vitamin C (Aladdin, 99%) were added in to the studied cells in plateau phase of growth (>90% coverage rate). As poor water solubility, polyphyllin was dissolved in DMSO [23]. High-purity synthetic air (>99.999%), high-purity nitrogen (>99.999%) and carbon dioxide (>99.999%) were purchased from Beijing Haikeyuanchang Practical Gas Co. Ltd. and Beijing Huayuan Gas Chemical Industry Co. Ltd, respectively.

2.4. Statistical analyses

SPSS version 20 statistical software (SPSS Inc), OriginPro 2015 software (OriginLab, Northampton, MA), Excel (Microsoft Excel, Excel 2013, Microsoft Corporation, USA) were used for data analyses.

Preliminary pairwise comparison of VOCs mass spectrometric profiling from all cell lines was conducted in OriginPro, distinctive (increasing or decreasing) peaks were picked out. Further statistic was performed by other software. Normally distributed data is provided as mean ± standard deviation (SD). A two-sided non-parametric Mann-Whitney U-Test was used to identify substantial disparities between two groups of unpaired data. A multifactorial analysis of variance (ANOVA) was performed to examine the change of measured values over time per cell-line group. P-values < 0.05 were considered statistically significant for all statistical tests.

2.5. Metabolic pathway building using KEGG

The bioinformatics prediction based on available biochemical knowledge were made using the “Kyoto Encyclopedia of Genes and Genomes” (KEGG), available in http://www.genome.jp/kegg/ (Kanehisa Laboratory, Kyoto University). The identified emanations were matched with KEGG bioinformatics database and the compounds in common for all possible pathway pairs were found.

3. Results

3.1. Interpretation of raw spectra and identification of cancerous cells

As an accessible technic to identify cancer-related VOCs, recognizable spectra for these VOCs biomarkers should be obtained by the instrument. Initially, a continuous sampling with 10 s of acquisition time was employed for every cell line. As the headspace of culture medium containing high concentration of VOCs after several days of cultivation, the ion signals fluctuate too dramatically to be reliable at the beginning of each measurement. The actual concentration of certain compound should be given by the continuous exchange between the imported synthetic air and culture medium after 20 min. As shown is [Supplementary Figure S1], each cell line (more than 90% coverage rate) and DMEM medium were continuous measured in 30 min repeatedly, and the concentration changes of most distinctive compounds (m/z 65, 83, 93, 101,111) between cancerous and non-cancerous cell lines were displayed. In the ten minutes period ion signals change severely with large error bars. On the contrary, ion signals after 15 min tend to be constant and credible, which means balanced and sustainable gas-fluid exchanges arrived in the culture flasks. The further

VOCs profiling comparison and statistical analyses were based on the 20-30 minutes stable phase data.

The spectra of cancerous cell lines were compared with non-cancerous cell line's respectively [Supplementary Figure 2], the mass peaks at 19, 37, 55 and 73Th (mass-to-charge ratio, m/z) were contributed from H_3O^+, $(H_2O)_2H^+$, as well as $(H_2O)_3H^+$ (protonated water clusters). Other significant dissimilar peaks were picked out for statistical analysis [Supplementary Tab. S1]. More visually, significant levels of twenty identified representative signal peaks were displayed as heat map [Figure 2], navy blue was used to represent the signals of normal cell line control (293 T), warmer color stand for cancerous cell line which is more identifiable from non-cancerous one. Apart from a few isolated cases, most of cancerous cell lines can be distinguished from normal one by the twenty VOC biomarkers.

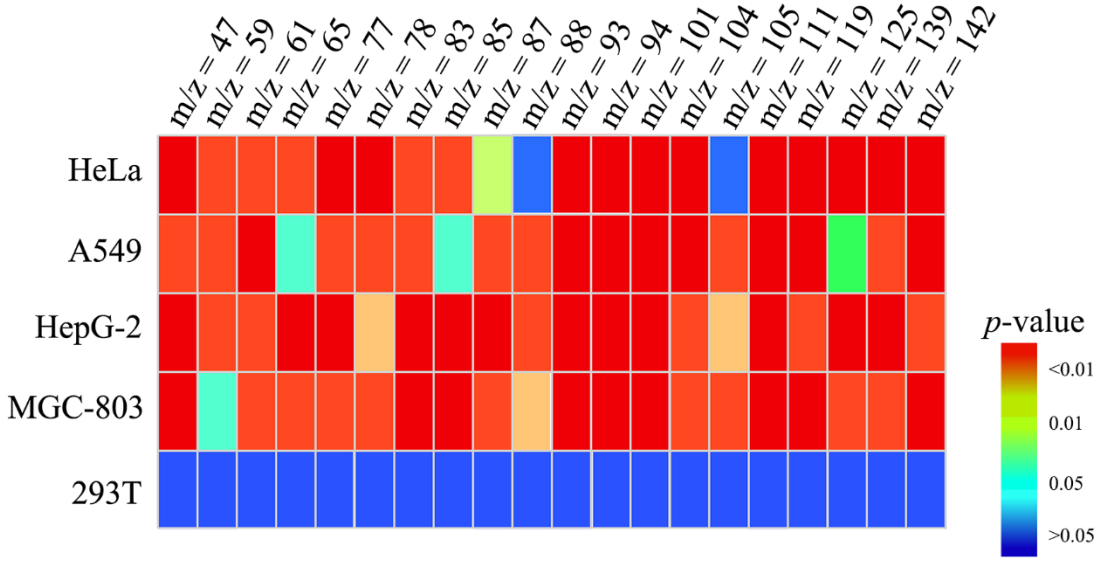

Figure 2. Significant levels of twenty biomarker candidates' peaks were displayed as heat map. In the bottom-right p-value scale, "0.01" and "0.05" correspond to a certain color respectively. "<0.01" and ">0.05" represent a section of color gamut respectively

In order to get an intuitive glimpse of the mass spectra, [Figure 3] shows the average concentrations obtained from three measurements and the corresponding standard deviations of VOCs emitted from four cancerous cell lines and non-cancerous control. The m/z range from 80 to 95 was chosen for it include more than a quarter of the twenty VOC biomarkers intensively. As can be seen, the measured signal intensities varied by two orders of magnitude from several hundreds to tens of thousands. In the case of m/z=83, m/z=91, m/z =93and m/z=94, the signals of cancerous cell lines were apparently higher than the non-cancerous one. As the VUV photoionization is softer than the electron impact ionization. The mass spectra of the VOCs obtained with the VUV photoionization are usually fragment free, the mass-to-charge ratio just correspond to the molecular weight of a compound. The compound at m/z=83 was tentative to be 2-methylfuran [27][28]. As regards m/z=91, protonated water clusters will contribute to this peak, the potential compounds could not be accurately identified. Other VOC biomarkers of cancerous cell lines that can be predicted by previous researches were listed in [Supplementary Table S1].

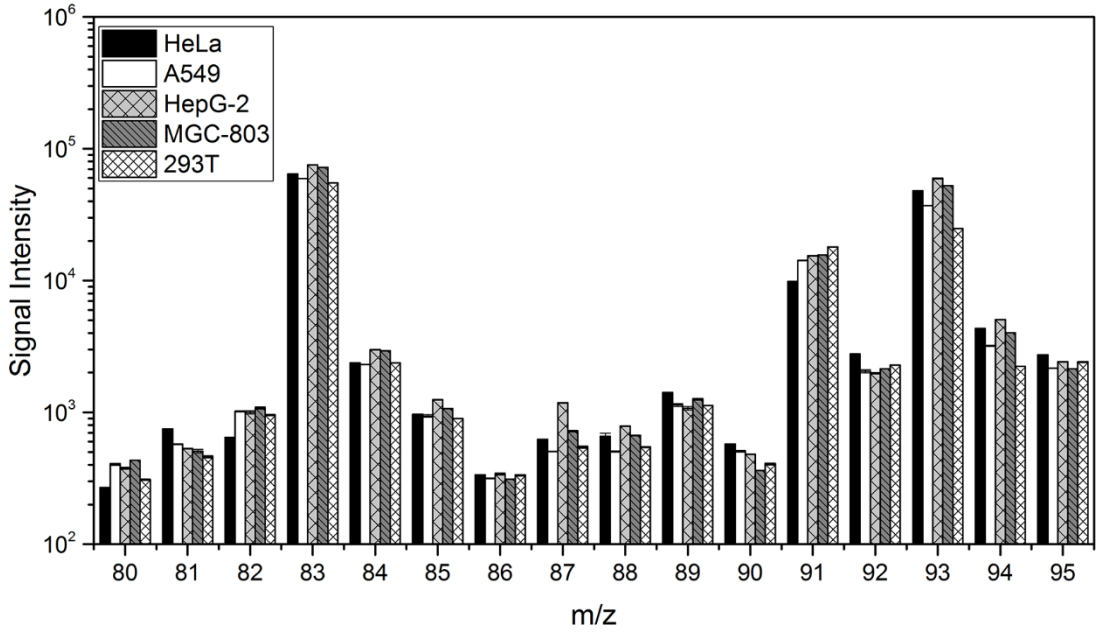

Figure 3. The average concentrations obtained from three measurements and the corresponding standard deviations of VOCs emitted from four cancerous cell lines and non-cancerous control within m/z range from 80 to 95

3.2. Polyphyllin effects on the VOCs metabolism of tumor cell lines

The signaling index that can represent drug-triggered cytotoxic effects is looked forward to explore and screen certain drugs' specific pharmacological effects against cancers. Thus, the VOC profiles under cytotoxic exposure were measured. Polyphyllin belongs to the antimitotic class of agents which can promote tubulin polymerization and is now widely used to treat a range of malignancies (19-21). Vitamin C was also included in this study as a positive control compound as it is always used in chemotherapy for its antioxidant (22, 23). After one-day treatment, the cytotoxic effects of polyphyllin exposures (50nM) were obvious, all of these cell lines were killed to less than 10% coverage rate. On the contrary, the vitamin C treated control was not inhibited at all, nearly 100% coverage rate were observed [Supplementary Table S2]. Recognizable signals at m/z=62 were observed in the cases of polyphyllin exposure. Especially for those cancerous cell lines, the mass peaks at 62 Th reached more than 100000 counts, substantially surpass the normal cell line exposure group [Figure 4]. No corresponding signal was observed in the DMEM medium with polyphyllin control.

The compound at m/z=62 was considered to be dimethyl sulphide (DMS) [17]. To further confirm and quantify, 10 ppb diluted DMS pure sample was injected in to a 120 L calibration chamber then measured by VUV-PIMS. The mass peaks at 62 and 47 Th were contributed by the molecular ion and single daughter ion of DMS [Supplementary Figure S3 a]. The linear calibration curve of DMS with a linear response (R^2 = 0.9992) was presented in [Supplementary Figure S3 b]. The linear regression equation of this curve was y = 997x + 10.3, suggesting a detection sensitivity of ~1 counts/pptv. According to the σ values obtained from the baseline of indoor air [Supplementary Figure S4 a] and liquid nitrogen trap purified high-purity nitrogen (Supplementary Figure S4 b), the limit of detection of DMS in ambient air (LOD_{air}) and limit condition (LOD_{lim}) were 3.72 pptv and 1.38 pptv, respectively. The

LOD of this instrument was more excellent than those of GC-MS and PTR-MS, 50 pptv and 25 pptv, respectively [29][30].

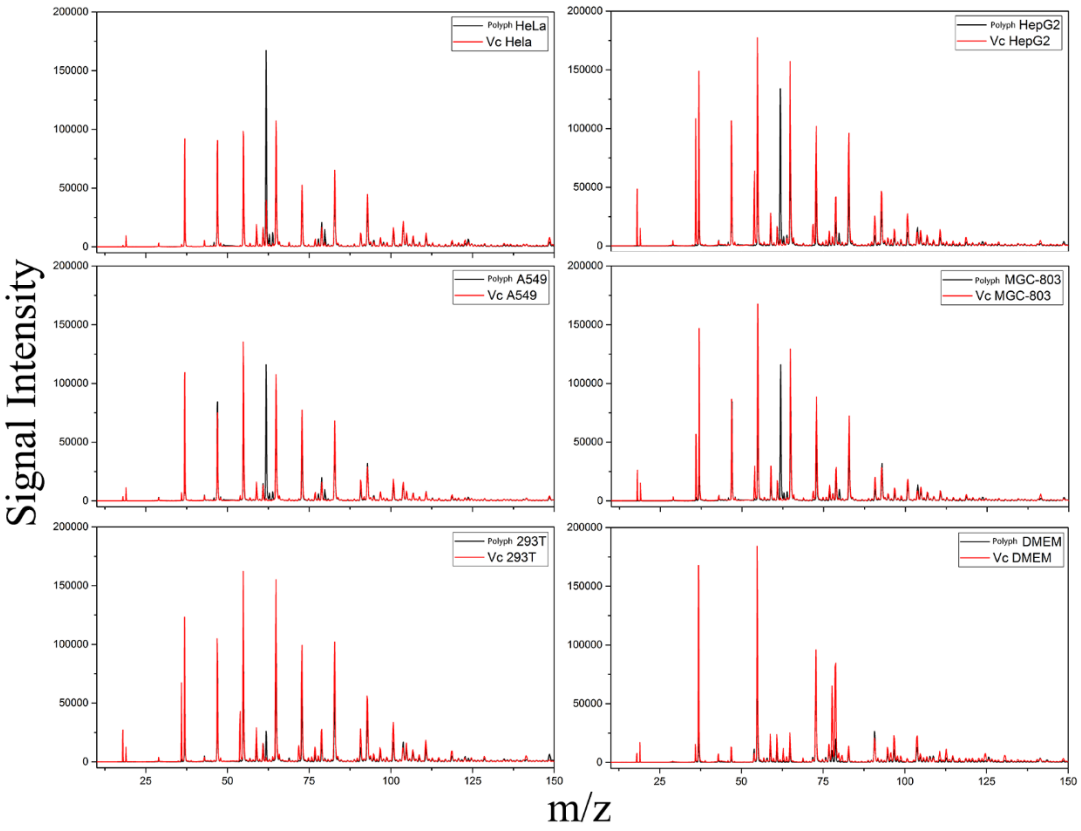

Figure 4. Mass spectrograms of each cell line after exposure to polyphyllin comparing with the ones exposed to Vc. Substantially high signals at m/z=62 were observed in the cases of polyphyllin exposure. Especially in those cancerous cases, the DMS concentration arrived to about 100 ppbv, significantly surpass the normal cell line exposure group.

Taking account that cancer development is always linked to boosted oxidative stress [3] and vitamin C is also used in chemotherapy for its antioxidant. The VOCs metabolism of each cell line after exposure for 24h to 50nM vitamin C was measured as well. As shown in [Supplementary Figure S5], no specific peaks were detected in the vitamin C exposure group. The VOCs profiling of vitamin C exposure group just small degree higher than the no-exposure ones generally. It might be derived from the longer culture time without cytotoxic effects.

The potential pathway by which so much DMS was released was proposed in [Figure 5]. DMS is anticipated to be the signature for the cytotoxic effects on cancerous cells. Considering the substantial variance of DMS in the polyphyllin exposure cancerous cell lines and non-cancerous cell control, the detection performance of VUV-PIMS might satisfies the demand of pharmacological screening of anti-cancer drugs.

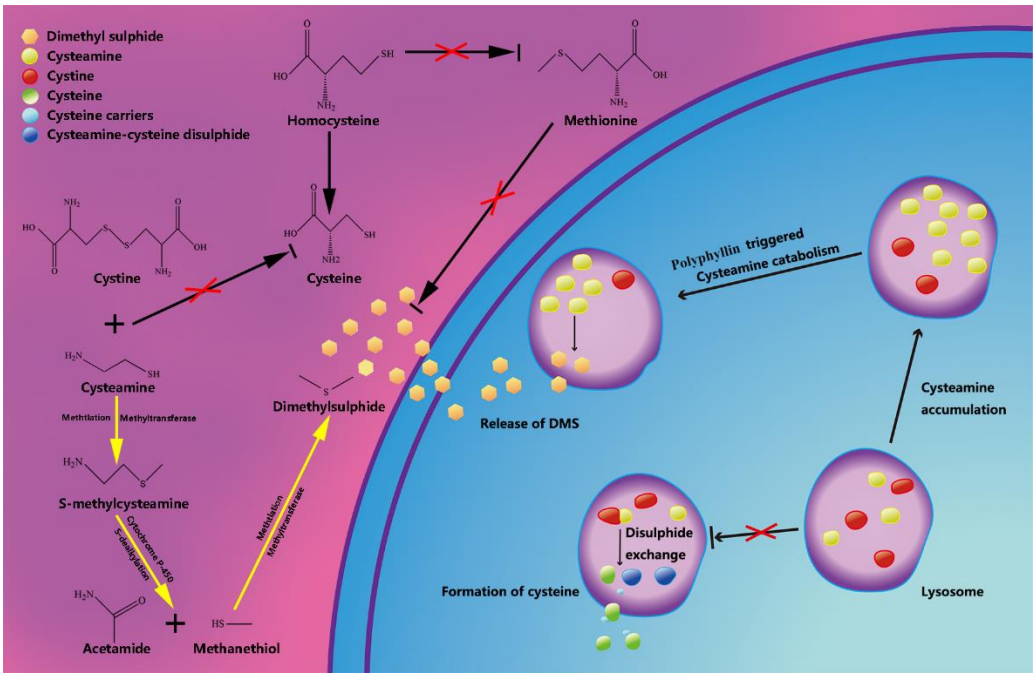

Figure 5. The hypothesized mechanism by with such large amount of DMS was released from cancer cells after polyphyllin exposure

3.3. Metabolic modeling

The specific VOC profiles of cancer cells depend on the abnormal pathway regulation, therefore, VOCs identified as biomarkers between cancerous and non-cancerous cell lines as well as the specific VOC released in polyphyllin exposure group were matched with the KEGG Pathway database, obtaining 5 relative metabolic pathways. A particular algorithm [31] was used for the putatively interconnected pathway [Figure 6].

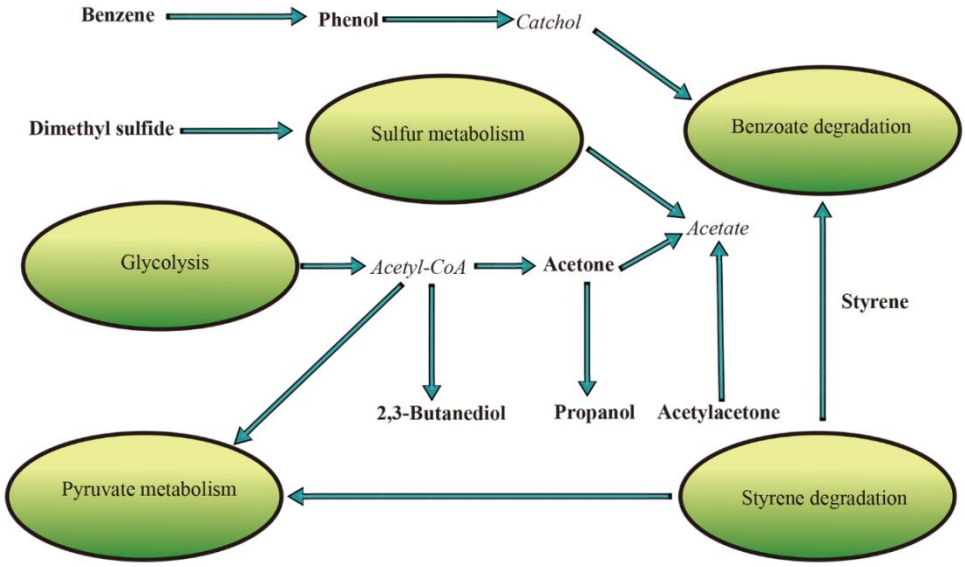

Figure 6. The possible pathways in common and including those tentative emanations identified by VUV-PIMS as cancer biomarkers were constructed

Apart from those compounds with the same molecular mass, others that can be identified by VUV-PIMS were displayed as bold fonts in [Figure 6]. The metabolic pathways were linked through a central branch mediated by pyruvate metabolism, indicating that energy related metabolisms had vital role in the volatile production of each cell line. Benzene, phenol and styrene were associated with benzoate degradation. Acetone, 2, 3-butanediol and propanol were involved in glycolysis. Styrene degradation can be linked with pyruvate metabolism and benzoate degradation simultaneously. Moreover, as a specific volatile compound released from polyphyllin exposure cell lines, dimethyl sulphide participate in sulfur metabolism.

4. Discussion

Pathophysiology demonstrate that cancer is associated with metabolic illnesses, abnormal processes alter the cells' chemistry by changing the VOCs concentration or by producing new VOCs. Cancer VOCs originate from the cells or disease location, enter the surrounding environment and can be identified from the headspace of cancer cell lines. Most of these volatile metabolites at low concentrations that range from pptv to ppbv. In this study, our newly developed ultrasensitive VUV-PIMS was used for uncovering cancer-related VOC biomarkers. This technique allows real-time detection and quantification of trace amount of VOCs emitted by cells (tissue) without any sample pre-concentration. The volatile candidates' assessment of four cancerous cell lines and one non-cancerous cell line revealed twenty metabolites' relative quantitation were able to distinguish normal from malignant cells. The combinative feature of these metabolites was strongly associated with all species of cancerous cell lines in this study, instead of certain species of cancer cell line. Thus, we propose that the measurement of these metabolites by VUV-PIMS could be useful in guiding intraoperative decisions of a wide variety of cancers. . As the detectable VOC biomarkers by VUV-PIMS and PTR-MS depend on the ionization energy and water affinity of molecular respectively, different cancer-related VOC profiles were acquired. Fortunately, the discrimination levels of VUV-PIMS measured VOC cancer-related biomarkers was universally higher than PTR-MS ones. Further study is anticipated to translate the lab results to clinical tools that could real-time recognize tumor margins from normal surrounding in vivo, during surgery.

According to the metabolic modeling, energy related metabolisms play substantial roles in the production of these volatile compounds, which is coherent with the consensus that cancer cells have a higher energy requirement compared with normal cells [32]. Moreover, because of the elevated demand for energy and macromolecular biosynthesis these cells prefer the use of glycolysis over oxidative phosphorylation (Warburg effect) [33][34].

Of note, markedly higher levels of dimethyl sulphide was observed in polyphyllin exposure cell lines, in particular cancerous ones. This phenomenon is of special interest, as it has not been previously reported. Previously, DMS was found to be substantially higher in alveolar air of patients with liver diseases and human hepatocellular carcinoma cells (HepG2)[35]. Exogenously, DMS has no significant effect on detoxification enzymes involved in carcinogen metabolism [36]. Due to its neutral nature, DMS is stable in blood and does not react with proteins. Free DMS is detected in blood of normal persons in low concentrations (<7 nM), approximate to the GC-MS detection limit [37]. Given the diffusion of DMS from cells (tissue) to the surrounding air, extremely lower concentration can be detected from the headspace of related tissue. Fortunately, our VUV-PIMS acquire the ultra-low detection limit of 3.7 pptv for DMS in ambient air.

Generally, sulphur-contained compounds are generated by incomplete metabolism of methionine in the transamination pathway, where mercaptans are easily oxidized to their respective sulphides [38][39]. However, cancer cells are characterized for their methionine dependence, whereas normal cells are usually methionine independent [40][41]. Homocysteine is the metabolic precursor for methionine synthesis [42] and can be converted to cysteine [43]. As methionine synthesis is blocked in cancer cells, higher level of cysteine is anticipated to produce. Besides, cysteamine removes cystine from lysosomes via a disulphide exchange reaction with cystine, resulting in the formation of cysteine which exit the lysosome via the cysteine carriers. Such approach might be negatively regulated by the high level of cysteine and lack of cysteine carriers. Therefore, cysteamine is accumulated in lysosomes. According to the above, we speculate that the DMS released from those polyphyllin exposure cell lines derived from a hypothesized route of cysteamine catabolism into DMS [44], which might be triggered by the apoptosis or cell death resulted from polyphyllin exposure [45]. More cysteamine enriched in cancerous cell lines, when confronted with cytotoxic effects (apoptosis and autophagy) more DMS was produced from the cancer cells by cysteamine catabolism. Conversely, the considerable production of DMS consumed a large amount of cysteamine, result in cysteine become relatively higher, which may positive regulate a the apoptosis of cancer cells [46]. This distinctive signal of DMS might serve as a therapeutic indicator in clinical practice, pending future studies further addressing the mechanism of DMS generation and pharmacological causal relationship to other kinds of anti-cancer drugs. This specific signal even can be used to evaluate the pharmacodynamics of antineoplastic agent candidates.

Acknowledgments

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References

[1] F. Kamangar, G.M. Dores, and W.F. Anderson, “Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world,” Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, vol.24, pp.2137-2150, **(2006)**

[2] D.T. Ross, U. Scherf, M.B. Eisen, C.M. Perou, C. Rees, P. Spellman, V. Iyer, S.S. Jeffrey, M. Van de Rijn, M. Waltham, A. Pergamenschikov, J.C.E. Lee, D. Lashkari, D. Shalon, T.G. Myers, J.N. Weinstein, D. Botstein, and P.O. Brown, “Systematic variation in gene expression patterns in human cancer cell lines,” Nature Genetics, vol.24, pp.227-235, **(2000)**

[3] H. Haick, Y.Y. Broza, P. Mochalski, V. Ruzsanyi, and A. Amann, “Assessment, origin, and implementation of breath volatile cancer markers,” Chemical Society Reviews, vol. 43, pp.1423-1449, **(2014)**

[4] Y. Hanai, K. Shimono, K. Matsumura, A. Vachani, S. Albelda, K. Yamazaki, G.K. Beauchamp, and H. Oka, “Urinary volatile compounds as biomarkers for lung cancer,” Bioscience, biotechnology, and biochemistry, vol.76, pp.679-684, **(2012)**

[5] R.Y. Xue, L. Dong, S. Zhang, C.H. Deng, T.T. Liu, J.Y. Wang, and X.Z. Shen, “Investigation of volatile biomarkers in liver cancer blood using solid-phase microextraction and gas chromatography/mass spectrometry,” Rapid Communications in Mass Spectometry, vol.22, pp.1181-1186, **(2008)**

[6] M. Goldoni, D. Poli, A. Caglieri, G. Ceresa, O. Acampa, P. Carbognani, M. Rusca, and M. Corradi, "Breath analysis in non small cell lung cancer patients after surgical tumour resection," Age (median, years), vol.67, pp.64-72, **(2008)**

[7] X. Chen, F. Xu, Y. Wang, Y. Pan, D. Lu, P. Wang, K. Ying, E. Chen, and W. Zhang, "A study of the volatile organic compounds exhaled by lung cancer cells in vitro for breath diagnosis," Cancer, vol.110, pp.835-844, **(2007)**

[8] G. Peng, U. Tisch, O. Adams, M. Hakim, N. Shehada, Y.Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten, and H. Haick, "Diagnosing lung cancer in exhaled breath using gold nanoparticles," Nature Nanotechnology, vol.4, pp.669-673, **(2009)**

[9] S. Fischer, P. Trefz, A. Bergmann, M. Steffens, M. Ziller, W. Miekisch, J.S. Schubert, H. Kohler, and P. Reinhold, "Physiological variability in volatile organic compounds (VOCs) in exhaled breath and released from faeces due to nutrition and somatic growth in a standardized caprine animal model," Journal of Breath Research, vol.9, pp.027108, **(2015)**

[10] J. Li, Y.L. Peng, and Y.X. Duan, "Diagnosis of breast cancer based on breath analysis: An emerging method," Critical Reviews in Oncology/Hematology, vol.87, pp.28-40, **(2013)**

[11] G. Peng, M. Hakim, Y.Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten, U. Tisch, and H. Haick, "Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors," British Journal of Cancer, vol.103, pp.542-551, **(2010)**

[12] T. Fink, J.I. Baumbach, and S. Kreuer, "Ion mobility spectrometry in breath research," Journal of Breath Research, vol.8, pp.027104, **(2014)**

[13] J. Balog, L. Sasi-Szabo, J. Kinross, M.R. Lewis, L.J. Muirhead, K. Veselkov, R. Mirnezami, B. Dezso, L. Damjanovich, A. Darzi, J.K. Nicholson, and Z. Takats, "Intraoperative tissue identification using rapid evaporative ionization mass spectrometry," Science Translational Medicine, vol.5, pp.194ra193, **(2013)**

[14] G. Konecny, M. Untch, D. Slamon, M. Beryt, S. Kahlert, M. Felber, E. Langer, S. Lude, H. Hepp, and M. Pegram, "Drug interactions and cytotoxic effects of paclitaxel in combination with carboplatin, epirubicin, gemcitabine or vinorelbine in breast cancer cell lines and tumor samples," Breast Cancer Research and Treatment, vol.67, pp.223-233, **(2001)**

[15] J. Wu, Y.H. Lu, A. Lee, X.G. Pan, X.J. Yang, X.B. Zhao, and R.J. Lee, "Reversal of multidrug resistance by transferrin-conjugated liposomes co-encapsulating doxorubicin and verapamil," Journal of Pharmacy and Pharmaceutical Sciences, vol.10, pp.350-357, **(2007)**

[16] W. Filipiak, A. Sponring, T. Mikoviny, C. Ager, J. Schubert, W. Miekisch, A. Amann, and J. Troppmair, "Release of volatile organic compounds (VOCs) from the lung cancer cell line CALU-1 in vitro," Cancer Cell International, vol.8, pp.17, **(2008)**

[17] P. Mochalski, A. Sponring, J.L. King, K. Unterkofler, J. Troppmair, and A. Amann, "Release and uptake of volatile organic compounds by human hepatocellular carcinoma cells (HepG2) in vitro," Cancer Cell International, vol.13, **(2013)**

[18] A. Sponring, W. Filipiak, C. Ager, J. Schubert, W. Miekisch, A. Amann, and J. Troppmair, "Analysis of volatile organic compounds (VOCs) in the headspace of NCI-H1666 lung cancer cells," Cancer Biomarkers : section A of Disease markers, vol.7, pp.153-161, **(2010)**

[19] C. Brunner, W. Szymczak, V. Hollriegl, S. Mortl, H. Oelmez, A. Bergner, R.M. Huber, C. Hoeschen, and U. Oeh, "Discrimination of cancerous and non-cancerous cell lines by headspace-analysis with PTR-MS," Analytical and Bioanalytical Chemistry, vol.397, pp.2315-2324, **(2010)**

[20] T.J. Christian, "Comprehensive laboratory measurements of biomass-burning emissions: 2. First intercomparison of open-path FTIR, PTR-MS, and GC-MS/FID/ECD," Journal of Geophysical Research, vol.109, **(2004)**

[21] F. Muhlberger, R. Zimmermann, and A. Kettrup, "A mobile mass spectrometer for comprehensive on-line analysis of trace and bulk components of complex gas mixtures: Parallel application of the laser-based

ionization methods VUV single-photon ionization, resonant multiphoton ionization, and laser-induced electron impact ionization," Analytical Chemistry, vol.73, pp.3590-3604, **(2001)**

[22] F. Muhlberger, J. Wieser, A. Ulrich, and R. Zimmermann, "Single photon ionization (SPI) via incoherent VUV-excimer light: Robust and compact time-of-flight mass spectrometer for on-line, real-time process gas analysis," Analytical Chemistry, vol.74, pp.3790-3801, **(2002)**

[23] J.E. Liebmann, J.A. Cook, C. Lipschultz, D. Teague, J. Fisher, and J.B. Mitchell, "Cytotoxic studies of paclitaxel (Taxol) in human tumor-cell lines," British Journal Of Cancer, vol.68, pp.1104-1109, **(1993)**

[24] C.M. Kurbacher, U. Wagner, B. Kolster, P.E. Andreotti, D.Krebs, and H.W. Bruckner, "Ascorbic acid (vitamin C) improves the antineoplastic activity of doxorubicin, cisplatin, and paclitaxel in human breast carcinoma cells in vitro," Cancer letters, vol.103, pp.183-189, **(1996)**

[25] J.N.Shu, W.Q. Sun, P. Zhang, Z. Li, N. N. Li, M. Liang, and B. Yang, "Real-time monitoring of trace-level VOCs by an ultrasensitive compact lamp-based VUV photoionization mass spectrometer," Atmospheric Measurement Techniques Discussions, vol.8, pp.5877-5894, **(2015)**

[26] X. Shu, B. Yang, J.W. Meng, Y.F. Wang and J.N. Shu, "Vacuum ultraviolet photoionization mass spectra of typical organics contained in ambient aerosols," Spectroscopy Letters, vol.46, pp.227-234, **(2013)**

[27] T. Ligor, L. Pater, and B. Buszewski, "Application of an artificial neural network model for selection of potential lung cancer biomarkers," Journal of Breath Research, vol.9, pp.027106, **(2015)**

[28] J. Rudnicka, T. Kowalkowski, T. Ligor, and B. Buszewski, "Determination of volatile organic compounds as biomarkers of lung cancer by SPME-GC-TOF/MS and chemometrics," Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences, vol.879, pp.3360-3366, **(2011)**

[29] P. Mochalski, J. King, M. Klieber, K. Unterkofler, H. Hinterhuber, M. Baumann, and A. Amann, "Blood and breath levels of selected volatile organic compounds in healthy volunteers," Analyst, vol.138, pp.2134-2145, **(2013)**

[30] A. Jordan, S. Haidacher, G. Hanel, E. Hartungen, J. Herbig, L. Mark, R. Schottkowsky, H. Seehauser, P. Sulzer, and T.D. Mark, "An online ultra-high sensitivity Proton-transfer-reaction mass-spectrometer combined with switchable reagent ion capability (PTR+SRI-MS)," International Journal of Mass Spectrometry, vol.286, pp.32-38, **(2009)**

[31] C.A. Acevedo, W. Creixell, C. Pavez-Barra, E. Sánchez, F. Albornoz, and M.E. Young, "Modeling volatile organic compounds released by bovine fresh meat using an integration of solid phase microextraction and databases," Food and Bioprocess Technology, vol.5, pp.2557-2567, **(2011)**

[32] R.A. Cairns, I.S. Harris, and T.W. Mak, "Regulation of cancer cell metabolism," Nature reviews, Cancer, vol.11, pp.85-95, **(2011)**

[33] J.W. Kim and C.V. Dang, "Cancer's molecular sweet tooth and the Warburg effect," Cancer Research, vol.66, pp.8927-8930, **(2006)**

[34] M.G. Vander Heiden, L.C. Cantley, and C.B. Thompson, "Understanding the warburg effect: the metabolic requirements of cell proliferation," Science, vol.324, pp.1029-1033, **(2009)**

[35] B. Buszewski, A. Ulanowska, T. Ligor, M. Jackowski, E. Klodzinska, and J. Szeliga, "Identification of volatile organic compounds secreted from cancer tissues and bacterial cultures," Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences, vol.868, pp.88-94, **(2008)**

[36] M.H. Siess, A.M. LeBon, M.C. CanivencLavier, and M. Suschetet, "Modification of hepatic drug-metabolizing enzymes in rats treated with alkyl sulfides," Cancer Letters, vol.120, pp.195-201, **(1997)**

[37] A. Tangerman, "Measurement and biological significance of the volatile sulfur compounds hydrogen sulfide, methanethiol and dimethyl sulfide in various biological matrices," Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences, vol.877, pp.3366-3377, **(2009)**

[38] O. Weisser and S. Landa, "Sulphide catalysts, their properties and applications," Academia, Prague, **(1972)**

[39] W. Miekisch, J.K. Schubert, and G.F. Noeldge-Schomburg, "Diagnostic potential of breath analysis--focus on volatile organic compounds," Clinica chimica acta; international journal of clinical chemistry, vol.347, pp.25-39, **(2004)**

[40] R.M. Hoffman, "Altered methionine metabolism, dna methylation and oncogene expression in carcinogenesis - a review and synthesis," Biochimica et Biophysica Acta, vol.738, pp.49-87, **(1983)**

[41] J.O. Mecham, D. Rowitch, C.D. Wallace, P.H. Stern, and R.M. Hoffman, "The metabolic defect of methionine dependence occurs frequently in human-tumor cell-lines," Biochemical and Biophysical Research Communications, vol.117, pp.429-434, **(1983)**

[42] R.M. Hoffman and R.W. Erbe, "High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine," Proceedings of the National Academy of Sciences, U S A, vol.73, pp.1523-1527, **(1976)**

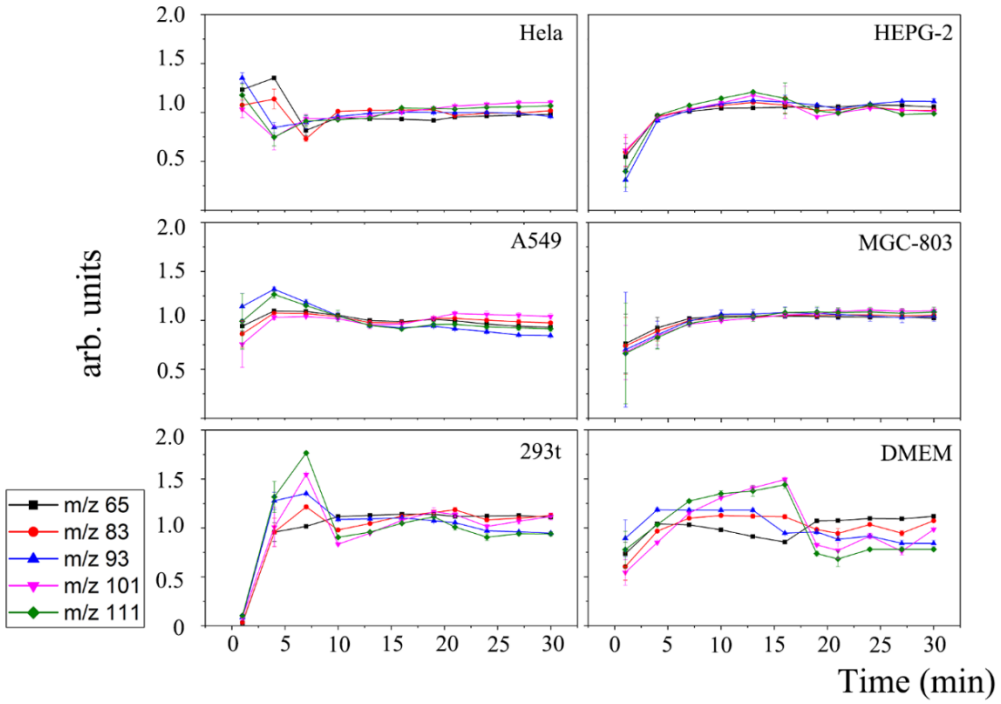
[43] N.H. Horowitz, "Methionine synthesis in neurospora - the isolation of cystathionine," Journal of Biological Chemistry, vol.171, pp.255-264, **(1947)**

[44] M. Besouw, H. Blom, A. Tangerman, A. de Graaf-Hess, and E. Levtchenko, "The origin of halitosis in cystinotic patients due to cysteamine treatment," Molecular genetics and metabolism, vol.91, pp.228-233, **(2007)**

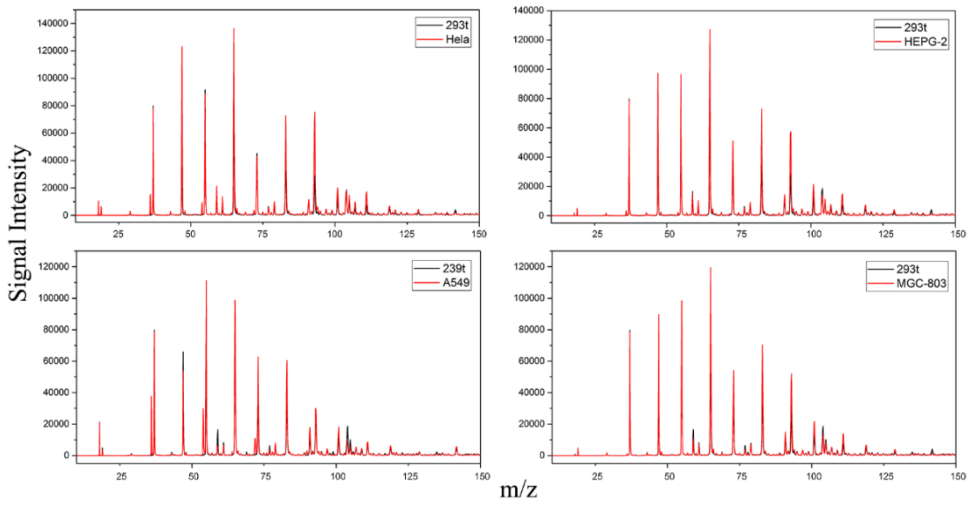
[45] M.A. Jordan, K. Wendell, S. Gardiner, W.B. Derry, H. Copp, and L. Wilson, "Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death," Cancer Research, vol.56, pp.816-825, **(1996)**

[46] M.A. Park and J.G. Thoene, "Potential role of apoptosis in development of the cystinotic phenotype," Pediatric nephrology, vol.20, pp.441-446, **(2005)**

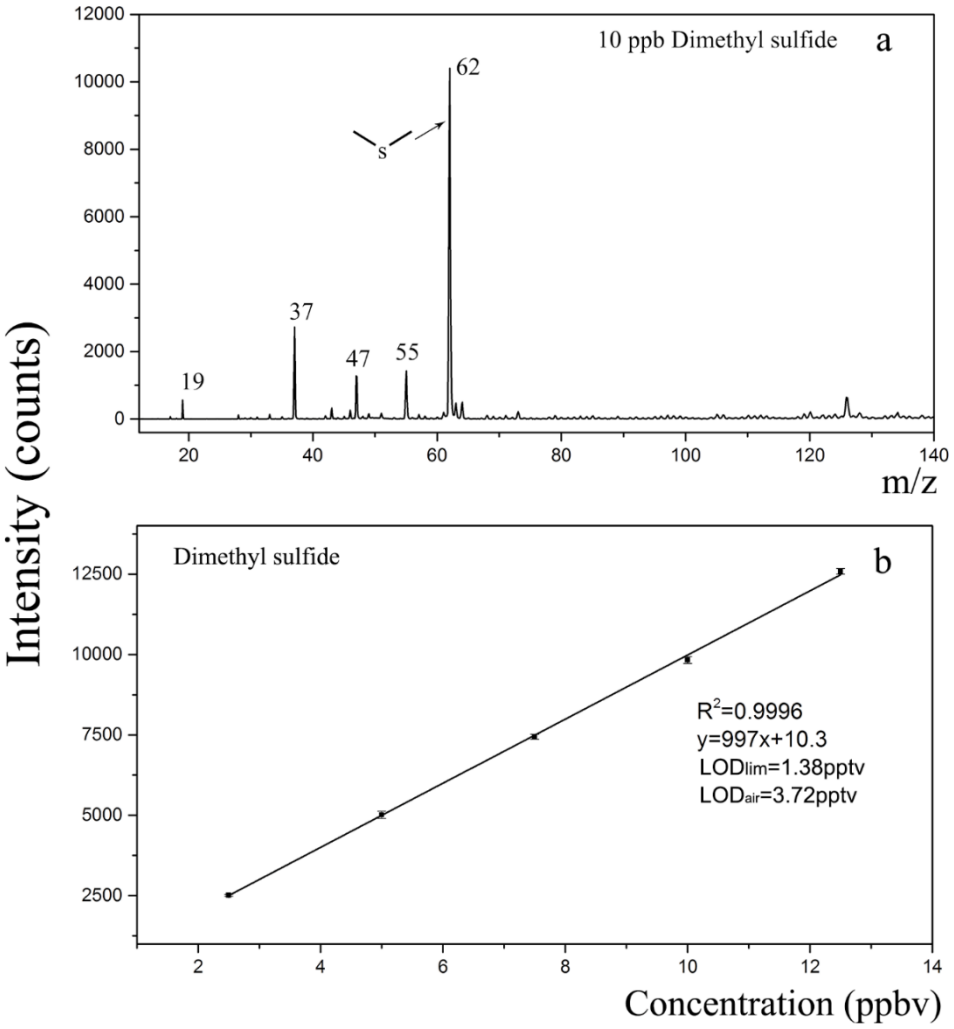
Appendix



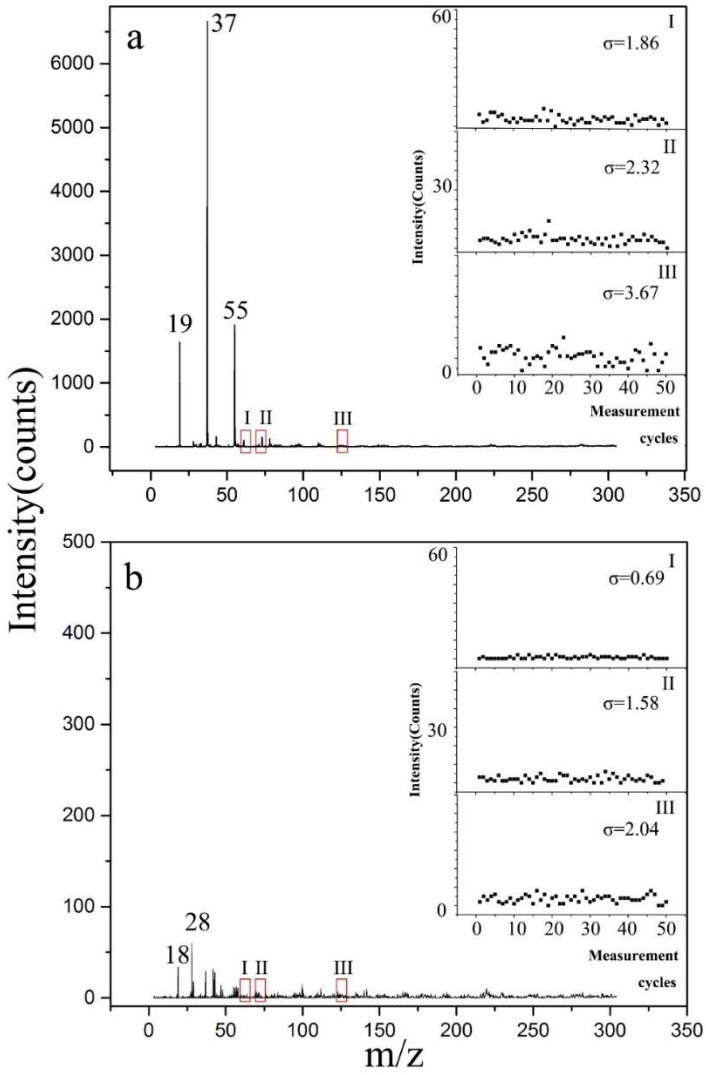
Supplementary Figure S1. Continuous measurement of the concentration changes of most distinctive compounds (m/z 65, 83, 93, 101,111) in 30 min repeatedly between cancerous and non-cancerous cell lines



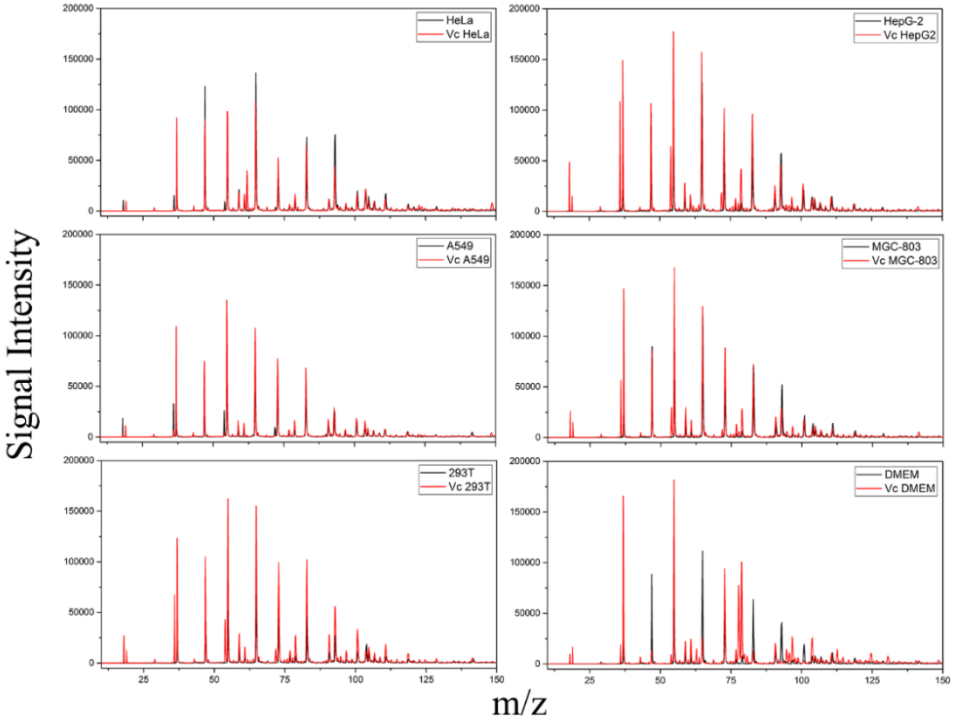
Supplementary Figure S2. The spectra of cancerous cell lines comparing with non-cancerous cell line's respectively



Supplementary Figure S3. 10 ppb diluted DMS pure sample injected in to a 120 L calibration chamber and measured by VUV-PIMS. b. The linear calibration curve of DMS and corresponding linear regression equation of this curve. (Zhen Li, Ji Shu, Peng Zhang, Wanqi Sun, Bo Yang and Haixu Zhang (2016) Real-time ultrasensitive VUV-PIMS detection of representative endogenous volatile markers in cancers. Cancer Biomarkers 16:477-487.)



Supplementary Figure S4. The baseline of indoor air measured by VUV-PIMS. b. The baseline of liquid nitrogen trap purified high-purity nitrogen by VUV-PIMS. (Zhen Li, Ji Shu,, Peng Zhang, Wanqi Sun, Bo Yang and Haixu Zhang (2016) Real-time ultrasensitive VUV-PIMS detection of representative endogenous volatile markers in cancers. Cancer Biomarkers 16:477-487.)



Supplementary Figure S5. The spectra of vitamin C exposure group comparing with the no-exposure group

Supplementary Table S1.

	m/z	Hela Signal Intensity	A549 Signal Intensity	HEPG-2 Signal Intensity	MGC-803 Signal Intensity	293t Signal Intensity	p-Values	Tentative compound	Refer
Cancer-SpecificReleased VOCs	47	49084.3±127	52164±240.2	97222.3±96.6	89383.3±224.3	64586±136.6	-; -; <0.01; <0.01;	Etanol	
	65	92896.7±262.9	91445.3±174.3	127036±116.1	119507.3±57.4	90925.3±249.8	<0.01; 0.17; <0.01; <0.01;	Ethanol cluster ions	
	83	64617.7±288.6	59603.3±61.8	75378±54.3	72301.7±76	55226.7±112.9	<0.01; <0.01; <0.01; <0.01;	Methylfuran	
	85	964±8	939.3±22.7	1247.7±8.1	1068±5.2	896.7±2.1	0.01; 0.1; <0.01; <0.01;	Dichloromethae	
	87	619.7±6.5	505.3±3.1	1180.3±10.3	719.3±9.7	649±12.8	-; -; <0.01; <0.01;	2-Pentanone	
	88	657.7±37	505.3±6.1	786.7±7.5	669±5	611.3±8.1	0.16; <0.01; <0.01; 0.02	Ethyl acetate; 2-Methoxy-2-	

								methylpropane	
	94	4333.7±29	3200±28.5	5051.7±27	4022.7±14.6	2231±9.2	<0.01; <0.01; <0.01; <0.01;	-	
	101	20305±142.2	18572.7±33.6	20301.3±70.4	22532.3±15.6	15728.7±130.5	<0.01; <0.01; <0.01; <0.01;	Acetylacetone	
	111	17579.7±140	10134.7±29.9	13246.7±53.8	14539.7±54.1	7196±70.2	<0.01; <0.01; <0.01; <0.01;	Methylfurfural	
	119	7207±38.6	6012.3±90.5	6353±51.2	7262.3±28	5766.3±28.2	<0.01; 0.05; <0.01; <0.01;	Trichloromethane	
	125	1868.3±3.1	1310±13.1	1738.3±5	1492±3.6	1349±15.6	<0.01; 0.06; <0.01; <0.01;	-	
	139	2264.3±11	1023±4.6	1542.3±5.5	1423±15.6	895±1.7	<0.01; <0.01; <0.01; <0.01;	-	

Supplementary Table S2.

Cell-Line	Surviving Fraction (Polyphyllin Exposure)	Surviving Fraction (Vc Exposure)	Signal Intensity of m/z=62 (Polyphyllin Exposure)	Signal Intensity of m/z=62 (Vc Exposure)
HeLa	0.09	1.44	167271	40502
A549	0.08	1.17	116784	2557
HepG-2	0.08	1.06	134686	6134
MGC-803	0.09	1.13	116810	3292
293T	0.08	1.12	26502	3928