Release of Volatile Organic Compounds from the Lung Cancer Cell Line NCI-H2087 *In Vitro*

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Abstract. *Aim: The aim of this work was to confirm the existence of volatile organic compounds (VOCs) specifically released by lung cancer cells. Materials and Methods: NCI-H2087 cells were trypsinized and 100×10^6 cells were incubated in a sealed fermenter overnight. Samples from the headspace of the culture vessel were collected with simultaneous preconcentration by adsorption on solid sorbents and subsequently thermodesorbed for analysis by gas chromatography mass spectrometry (GC-MS). Results: The results showed a significant increase in the concentration of 2-ethyl-1-hexanol and 2-methylpentane in the headspace as compared with medium controls. 2-Methylpentane is also found in exhaled breath of lung cancer patients in contrast to that from healthy volunteers. Statistically significantly lower abundances of acetaldehyde, 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and butyl acetate were found. Conclusion: Our findings demonstrate that certain compounds can be cancer cell derived and thus may be indicative of the presence of a tumor. Some compounds were not released but seem to be consumed by cancer cells.*

Analysis of exhaled breath for recognition of human diseases using endogenous volatile organic compounds (VOCs) offers the possibility of noninvasive diagnosis (1-4). It is an ambitious goal, but some interesting pilot results are already available, *e.g.* for lung and breast cancer (5-10). Examples of volatile compounds in exhaled breath are methanol, acetone, isoprene, ketones and aldehydes, as well as some hydrocarbons. Acetone is one of the most abundant compounds in exhaled breath, its concentration being higher in patients with uncontrolled diabetes mellitus (11). Sulphur-containing compounds such as ethylmercaptane, dimethylsulfide and dimethyldisulfide are responsible for the characteristic odour of cirrhotic patient's breath (12), whereas *N*-containing compounds are typical for the breath of patients suffering from uremia (13). Ethane and pentane were found in increased concentrations in connection with lipid peroxidation (14, 15).

Nevertheless, the cellular and biochemical origin of endogenous VOCs is only poorly known and has to be further elucidated. This is particularly important in cases where such substances may be used as disease markers, *e.g.* in cancer. Tumorous and non-tumorous cells, immune cells and infectious agents may all contribute to VOC production. Reliable results may be obtained by *in vitro* studies, looking at isolated components, *i.e.* cancer cell lines, immune cells or microbes. Only very few published papers investigated the release of VOCs from human cancer cells *in vitro* (16, 17), whereas immune-competent cells have not been investigated at all with respect to release of VOCs. Headspace on-line measurements by selected ion flow tube mass spectrometry (SIFT-MS) were able to detect acetaldehyde that was released from the lung cancer cell lines SK-MES and CALU-1 (16). Chen *et al.*, who applied gas chromatography-mass spectrometry (GC-MS) after solid-phase microextraction (SPME), found several volatile compounds in the headspace of the medium of different types of primary lung cancer cells which differed in VOC-release from control cells (17). In their work, cells from squamous cell carcinoma, adenocarcinoma, bronchioloalveolar carcinoma and non-small cell lung cancer

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were compared with medium control and control cells including taste-bud cells, osteogenic cells, lipocytes and bronchial epithelial cells. Another line of investigation refers to the degradation of tryptophan catalyzed by the enzyme indoleamine 2,3-dioxygenase (18) which could impair the function of the immune system in cancerogenesis (19, 20) and might result in the release of formaldehyde from immune and cancer cells *in vivo* and *in vitro*. There is evidence that formaldehyde release is increased in cancer patients (10). Spanel *et al*. investigated the release of VOCs from the urine of bladder and prostate patients in headspace analysis by SIFT-MS and increased concentrations of formaldehyde from cancer patients compared to healthy controls were found (21).

In the present work, we examined the release of VOCs from the lung cancer cell line NCI-H2087 after incubation of the cells in a sealed fermenter under controlled ventilation with pure air.

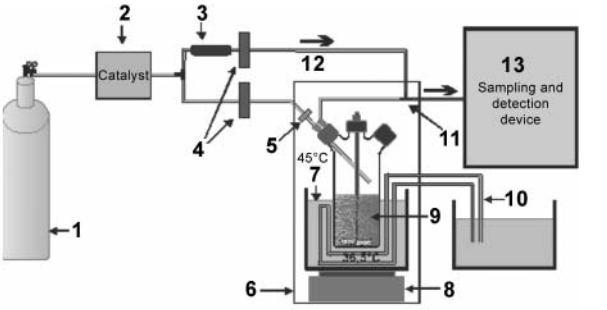

Figure 1. *Scheme of the device used for cell culture experiments and VOC analyses. Detailed scheme of the device for one fermenter (1) 50 l gas cylinder (5% CO_2, 95% synthetic air), (2) catalyst, (3) Supelcarb™ hydrocarbon trap, (4) thermal mass flow controller (measuring range 6-300 ml/h air for cell fermenter and 2-100 ml/min air for dilution), (5) sterile filter, (6) incubator, (7) water bath with heating circuit (36.5˚C), (8) magnetic stirrer with interval function, (9) cell fermenter, (10) electronically controlled valve, (11) dilution line, (12) diluent air, (13) sampling and detection devices.*

Materials and Methods

Cell culture. The lung tumor cell line NCI-H2087 (ATCC: CRL-5922; Promochem, Wesel, Germany) was grown in RPMI-1640 culture medium supplemented with 5% fetal calf serum (FCS), penicillin (100,000 units/l), streptomycin (100 mg/l) and L-glutamine (293 mg/l). Cells were cultivated under standard conditions in a conventional incubator at 37˚C in a humidified atmosphere with 92.5% air/7.5% CO_2. For VOC measurements 100×10^6 trypsinized cells were inoculated in 200 ml phenol red-free medium in a cell culture fermenter, which was flushed with clean, synthetic air taken from a gas cylinder (50 l defined gas mixture; Linde, Stadl-Paura, Austria) containing 5% CO_2 for at least 90 min and then sealed for 16 h. At the end of the incubation time, 200 ml of air from the headspace was used for GC-MS analyses. The sampled air was diluted 1:5 with dry and additionally purified synthetic air to avoid excess humidity for thermodesorption. Cell viability counts (trypan blue exclusion method) were performed at the end of the incubation period after 16 h.

Cell culture device. A sophisticated device for culturing cells under controlled conditions was developed (Figure 1). For ventilation of cells, air from a gas cylinder (50 l defined gas mixture; Linde) was used and an inline-catalyst (Parker Zero Air Generator, Balston®, Model: 75-83-220; Parker Hannifin Corporation, Haverhill, Massachusetts, USA) was installed to remove residual VOCs.

In this device, a constant and properly adjusted air flow through the system is controlled by a mass flow control unit (Model: F-200CV-005-AAD-11-V Multi-Bus DMFC (A-C); measuring range 0,1-5 ml/min air; Bronkhorst, Ruurlo, Netherlands). The mass flow control unit is connected to software which allows the control of flow settings on the flow controller as well as changes in the settings to be made. Following the flow control unit, air is conducted to a fermenter (Bellco®, Vineland, NJ, Canada) used for cell cultivation. In front of the fermenter, a sterile hydrophobic PTFE filter with 0.2 μm pore size (Sartorius, Göttingen; Germany) is placed to avoid contamination of the system. A Teflon tube then conducts air for sampling and analysis to a sorption tube or other sampling and measurement devices, respectively. Inside the fermenter, a magnetic Teflon stir bar is placed that is driven by a magnetic stirrer. To avoid condensation, the parts outside the water-bath are kept at a temperature of 45˚C. The water surrounding the cell fermenters has to be cooled to 36.5˚C to maintain an appropriate temperature for the cells. This is done by a cooling circuit operated and controlled by a water-bath (Julabo, Seelbach, Germany) placed outside the incubator.

For thermal desorption and also other sampling methods, sampled air must not be humidified to 100% because this would disturb the function of valves or cause a wide water peak in the chromatogram on GC-MS analysis. Thus, humidified, water-saturated air from cell culture must be diluted by dry air. For dilution of sampled air, a special flow controller (Bronkhorst, Ruurlo, Netherlands, Model: F-201CV-100-AAD-11-V Multi-Bus DMFC (D), measuring range: 2-100 ml/min air) with a higher range compared to flow controllers normally used for cell culture is needed. Moreover, a Supelcarb™ hydrocarbon trap is installed in front of the flow controller for dilution of air to ensure that impurities are removed completely. Further, an electronically controlled valve placed inside the incubator is used to conduct dilution air to the sampled air of any fermenter. This technical tool allows online measurements at any time from one of the used cell culture fermenters or adsorption on a thermal desorption tube.

Calibrations. For quantification of compounds detected in headspace of cells and medium solutions external standard calibration was performed. The following substances were used: 1-propanol, 2-methylpropanal, 2-butanone, 2-methylpentane, 3-methylpentane, methylcyclopentane, 3-methylbutanal, 2-methylbutanal, hexanal, n-butyl acetate, 2,3,4-trimethylpentane, 4-methylheptane, 2,3-dimethylheptane, 2-ethyl-1-hexanol (Sigma-Aldrich, Steinheim, Germany); 2,4-dimethylheptane, 2,3,3-trimethylpentane, 2,4-dimethyl-1-heptene and 4-methyloctane (ChemSampCo, LLC, Trenton, New Jersey, USA); acetaldehyde and 2,3-dimethylbutane (Acros Organics, Geel, Belgium). Preparation of gaseous standards was performed by evaporation of liquid substances in glass bulbs. Each bulb (Supelco, Bellefonte, PA, USA) was cleaned with methanol (Sigma-Aldrich), dried at 85˚C for at least 20 hours, purged with clean nitrogen for at

least 20 min and subsequently evacuated using a vacuum pump (Vacuubrand, Wertheim, Germany) for 30 minutes. Liquid standards (1-3 μl according to desired concentration) were injected through a septum using a GC syringe. After the evaporation of standards, the glass bulb was filled with nitrogen of purity 6.0 (*i.e.* 99.9999%, Linde, Vienna, Austria) in order to equalize the pressure (to the ambient pressure). The appropriate volume (μl) of vapor mixture was transferred using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) into Tedlar® bags (SKC 232 Series: Eighty Four, PA, USA) previously filled with 1.5 l of nitrogen (99.999%). For the preparation of retention time data, different concentrations of standard mixtures were used to avoid eventual problems by retention time shifting.

Sampling. Glass tubes (Gerstel, Mülheim an der Ruhr, Germany) filled with the following sorbents were used as traps for sample enrichment: 25 mg Tenax TA (60/80 mesh), 35 mg Carboxen 569 (20/45 mesh), 250 mg Carboxen 1000 (80/100 mesh) (each from Supelco). Each sorbent was separated from the next by glass wool. Because of the high humidity, sampled air was diluted with dry synthetic air (5% CO_2) at a ratio of 1:5. The volume of collected sample originating from the fermenter was 200 ml, with a total flow of 30 ml/min.

The sampled analytes were released from sorbents by thermal desorption in the TDS3 unit equipped with a TDSA2 auto sampler (both from Gerstel). The flow rate of carrier gas through the sorbent resin during desorption was 90 ml/min. The temperature ramp was as follows: The initial temperature was 30˚C and was increased to 300˚C at a rate of 100˚C/min (held for 10 min). Liquid nitrogen was used for cryofocusing the desorbed analytes (–90˚C). For subsequent sample injection into the capillary column, the injector (which contained the glass liner filled with Carbotrap B) was heated at 12˚C/s up to 320˚C (held for 2 min in splitless mode).

GC-MS analyses. Analyses were performed on an Agilent Technologies gas chromatograph, model 6890N equipped with mass selective detector 5973N (both from Agilent, Waldbronn, Germany). A 25 m × 0.32 mm × 5 μm PoraBond Q column was used (Varian, Palo Alto, CA, USA) and the following temperature program employed: 50˚C held for 5 min and followed by a temperature ramp of 5˚C/min up to 140˚C, which was held for 5 min. The temperature was then increased at a rate of 5˚C/min to 280˚C and held for 4 min. The helium flow rate was 2 ml/min. Ionization of separated compounds was performed by electron impact ionization at 70 eV. A TIC mode with full scan range of m/z=20 to m/z=200 was used. The acquisition of chromatographic data was performed by means of the Agilent Chemstation Software (GC-MS Data Analysis from Agilent, Waldbronn, Germany).

Results

Cell culture device and cultivation techniques. Considerable effort was invested to construct a cell culture device which is useful for headspace sampling. For these *in vitro* experiments, it was crucial that aeration of the cells in particular was strictly controlled and temperature is carefully adjusted. Our device allows sampling of headspace air by thermal desorption, SPME or direct sampling to other analytical devices. Here we used a simplified protocol that was already used by Smith and Spanel (22) (see Materials and Methods), with prolonged incubation of cells in the sealed cell culture fermenter for accumulation of VOCs.

Viability. In all experiments, it was important to ensure high cell viability since dying cells may cause changes in the composition of released VOCs. In GC-MS analyses, viability after inoculation of 100×10^6 cells after 16 h of culture was 88±4.4%. Thus, cell culture conditions did not cause substantial cell death.

GC-MS analyses. GC-MS analyses after preconcentration by adsorption on solid sorbents showed clear and reproducible differences in the concentrations of several compounds in the headspace of cells compared to headspace of medium control. The detected substances were identified by spectral match with the NIST 2005 spectral library (Gatesburg, PA, USA). However, this approach is very often misleading in the case of very low concentrations of analytes due to impurities of spectra from noise. To assure a maximum reliability of the results, the identification was carried out not only by spectral library match using NIST 2005 library: the presence of compounds suggested by NIST library was additionally verified by confirmation of their retention time. For this purpose, mixtures of pure reference materials were prepared following the same protocol which was described for calibration mixtures. The calibration measurements were used to determine the limit of detection (LOD) and subsequent quantification of the analytes found in the headspace of cell culture.

Results in Table I present the accuracy of the calibration regression line. Generally, the TD-GC-MS method applied is characterized by good linearity of the calibration lines (even for the lowest detected concentrations), with correlation coefficients being >0.99. The concentrations of reference materials were progressively reduced to estimate the correct limits of detection (LOD) of analytes. The lowest LODs were found for hexane (1.655×10^{-4} μg/l), pentane (1.86×10^{-4} μg/l), 2,3-dimethylheptane (4.903×10^{-4} μg/l), and 2-methylpentane (1.53×10^{-7} μg/l), while the highest LODs were found for 2-ethyl-1-hexanol (1.323 μg/l), 1-propanol (0.784 μg/l) and acetaldehyde (0.043 μg/l). More detailed information is presented in Table I. Detection limits as low as several tens of part per trillion unit (ppt_v) with simultaneous low errors (expressed by correlation coefficient) testify to the very good precision and sensitivity for certain groups of analytes.

The peaks of significantly altered substances are presented as average (n=3) peak areas (Figure 2 and Table II). Chromatograms for selected compounds are shown in Figure 3 A-C. The significance of the results was calculated by ANOVA.

In three independent experiments using GC-MS analyses after sample enrichment by adsorption on solid sorbents, the

Table I. *Summary of VOCs from NCI-H2087 cells analyzed by GC-MS after adsorption on solid sorbents and thermal desorption. Calibrations were performed, giving the calibration line and the limit of detection (LOD).*

Compound	CAS #	Retention time of standard (min)	Range of calibration (ppb)	Correlation coefficient, R^2 of standard curve	LOD (ppb)
Acetaldehyde	75-07-0	11.53	3.11-74.70	0.993±0.0043	2.54
1-Propanol	71-23-8	22.55	69.84-523.78	1±0.00017	79.63
Pentane	109-66-0	24.12	0.05-1.52	0.999±0.00091	0.07
2-Methylpropanal	78-84-2	25.47	2.76-69.01	0.997±0.0037	4.47
2-Butanone	78-93-3	27.32	0.98-19.57	0.999±0.00033	0.28
2,3-Dimethylbutane	79-29-8	31.21	0.67-13.45	0.999±0.00063	0.28
2-Methylpentane	107-83-5	31.42	0.33-6.65	0.999±0.00039	0.14
3-Methylpentane	96-14-0	31.9	0.67-13.47	0.999±0.00033	0.28
Methylcyclopentane	96-37-7	32.43	0.78-15.56	0.995±0.004	0.59
Hexane	110-54-3	32.83	0.05-1.50	0.999±0.00069	0.05
3-Methylbutanal	590-86-3	34.22	1.50-29.70	0.994±0.0026	0.44
2-Methylbutanal	96-17-3	34.38	2.34-58.45	0.994±0.0054	5.37
Hexanal	66-25-1	41.59	0.70-14.40	0.981±0.011	0.97
n-Butyl acetate	123-86-4	42.29	0.80-15.50	0.999±0.00029	0.24
2,3,4-Trimethylpentane	565-75-3	42.79	0.55-10.98	0.991±0.0052	2.32
2,3,3-Trimethylpentane	560-21-4	43.04	0.55-11.03	0.998±0.001	0.22
4-Methylheptane	589-53-7	43.21	0.54-10.77	0.985±0.0083	1.89
2,4-Dimethylheptane	2213-23-2	46.45	0.49-9.87	0.996±0.005	0.31
2,4-Dimethyl-1-heptene	19549-87-2	46.59	0.51-10.12	0.998±0.0018	0.19
2,3-Dimethylheptane	3074-71-3	47.37	0.49-9.88	0.999±0.00062	0.08
4-Methyloctane	2216-34-4	47.51	0.49-9.80	0.985±0.011	2.02
2-Ethyl-1-hexanol	104-76-7	51.21	335.80-503.60	0.843±0.034	113.87

Table II. *Summary of VOCs detected in headspace of NCI-H2087 cancer cells by means of GC-MS analysis after adsorption on solid sorbents and thermal desorption. The VOC concentrations are shown with the standard deviations. The p-values were calculated by ANOVA.*

Compound	CAS #	RPMI-1640 medium		NCI-H2087 cells		*p*-Values for Med *vs*. Cells (ANOVA)
		Average ppb	Stdev (ppb)	Average ppb	Stdev (ppb)	
Acetaldehyde	75-07-0	203.02	123.5	<LOD		0.02
1-Propanol	71-23-8	289.69	12.3	367.18	91.88	0.20
Pentane	109-66-0	0.29	0.09	1.17	0.67	0.08
2-Methylpropanal	78-84-2	8.02	0.8	5.19	0.22	<0.01
2-Butanone	78-93-3	17.66	1.25	21.25	2.01	0.06
2,3-Dimethylbutane	79-29-8	1.7	0.72	3.47	0.95	0.06
2-Methylpentane	107-83-5	1.84	0.55	6.56	2.61	0.04
3-Methylpentane	96-14-0	1.91	0.59	6.69	3.96	0.10
Methylcyclopentane	96-37-7	<LOD		9.85	7.18	0.08
Hexane	110-54-3	0.21	0.06	0.69	0.38	0.09
3-Methylbutanal	590-86-3	8.84	1.51	1.55	0.21	<0.01
2-Methylbutanal	96-17-3	14.15	1.05	8.2	0.1	<0.01
Hexanal	66-25-1	5.2	1.38	3.07	1.52	0.10
n-Butyl acetate	123-86-4	45.5	10.00	14.5	5.77	0.01
2,3,4-Trimethylpentane	565-75-3	3.29	0.12	5.65	2.41	0.20
2,3,3-Trimethylpentane	560-21-4	0.28	0.08	2.98	3.18	0.20
4-Methylheptane	589-53-7	2.25	0.18	5.71	2.9	0.10
2,4-Dimethylheptane	2213-23-2	4.74	1.37	9.9	4.66	0.10
2,4-Dimethyl-1-heptene	19549-87-2	3.21	0.84	6.68	3.12	0.10
2,3-Dimethylheptane	3074-71-3	1.61	0.56	3.43	1.8	0.20
4-Methyloctane	2216-34-4	16.52	6.76	36.59	16.66	0.10
2-Ethyl-1-hexanol	104-76-7	266.52	46.5	477.84	117.19	0.04

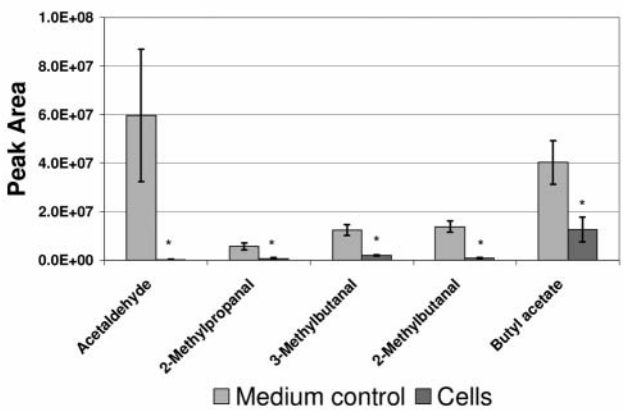

Figure 2. *Results of TD-GC-MS analyses of VOCs present at reduced concentrations in the headspace of NCI-H2087 cell culture as compared with medium only. Average peak areas (n=3) with standard deviation are shown. Medium control (bright columns) cell culture (dark columns). *Significantly different from the control at p≤0.02.*

concentrations of several substances were shown to be increased in the presence of NCI-H2087 cells (Figure 3 B-C and Table II). Concentrations of 2-methylpentane and 2-ethyl-1-hexanol were significantly increased, as expressed by *p*-values (0.04 for both) being below the threshold $p=0.05$. Other compounds showed an increase in some but not all of the experiments: this was the case for the hydrocarbons pentane (not significant = n.s.), hexane (n.s.), 2,3-dimethylbutane (n.s.), 3-methylpentane (n.s.), 4-methylheptane (n.s.), 2,4-dimethylheptane (n.s.), 2,3-dimethylheptane (n.s.) and 4-methyloctane (n.s.); the cyclic hydrocarbon methylcyclopentane (n.s.), and the unsaturated hydrocarbon 2,4-dimethyl-1-heptene (n.s.). From other classes of compounds, the alcohols 1-propanol (n.s.) and 2-ethyl-1-hexanol ($p<0.04$), as well as the ketone 2-butanone (n.s.), were increased in headspace from cells compared to the medium control. In the case of 1-propanol and 2-butanone, an increase was found in only two out of three experiments. Overall, only 2-methylpentane and 2-ethyl-1-hexanol showed a significant increase from cells as compared to medium controls. Reduced concentrations in the headspace of the tested cancer cell line compared to the medium control were observed for following compounds: acetaldehyde ($p<0.02$), 2-methylpropanal ($p<0.004$), 3-methylbutanal ($p<0.001$), 2-methylbutanal ($p<0.0006$), hexanal (n.s.) and butyl acetate ($p<0.01$), whereby significance was shown for all listed substances except for hexanal (Figure 2, 3 A-C and Table II).

Discussion

Identification and detection of VOCs that could provide for tumor markers in the breath of patients may finally provide a technically feasible method for early and noninvasive diagnosis of lung cancer. It is crucial to understand which

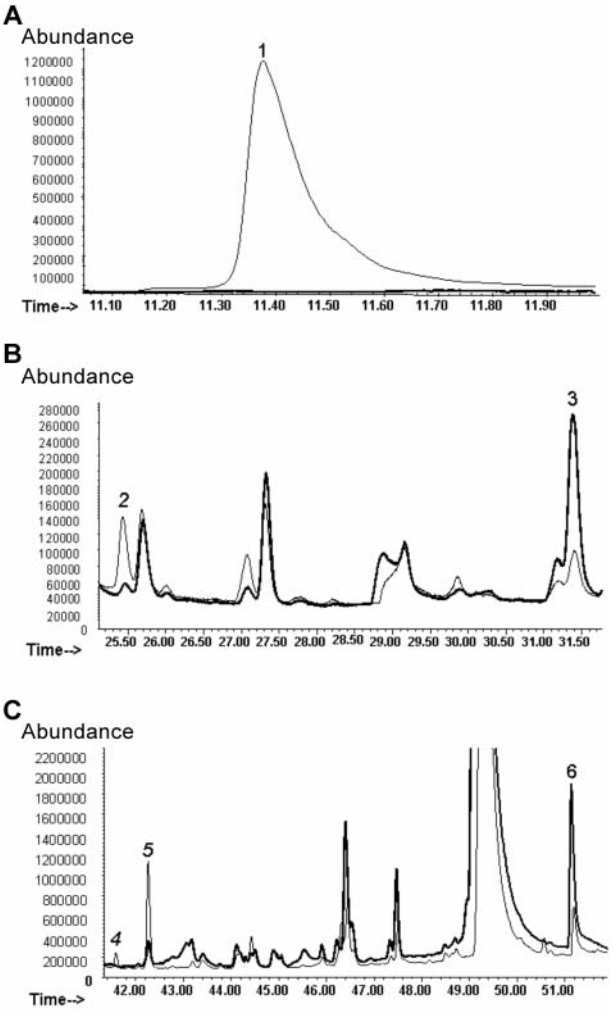

Figure 3. *TD-GC-MS Chromatograms of analyses of the headspace from NCI-H2087 lung cancer cell culture. A volume of 200 ml air from headspace diluted 1:5 with dry air was preconcentrated by means of multibed sorption traps and analyzed by GC-MS. Increased and decreased peaks, respectively, are numbered (decreased compounds in italic type). One representative result of three independent experiments (n=3, medium control in thin line) are shown. Identified substances: 1: acetaldehyde, 2: 2-methylpropanal, 3: 2-methylpentane, 4: hexanal, 5: butyl acetate, 6: 2-ethyl-1-hexanol.*

compounds are tumor-specific in order to use such VOCs as true tumor markers. In our experiments, the VOCs released from the lung cancer cell line NCI-H2087 were collected by adsorption on solid sorbents and after thermal desorption were analyzed by GC-MS.

For this study, a cell culture device and a sampling method for GC-MS which can be used for various experimental designs with various types of cells was developed. With this, it is possible to cultivate a high quantity of cells in a relative small cell culture vessel. Furthermore, because of the use of a sealed system and

absolutely pure air, reproducibility is possible, although the background signal from used culture media is rather high. Preconcentration of volatile compounds by use of multibed sorption tubes consisting of Tenax TA and Carbon molecular sieves (Carboxen 569 and Carboxen 1000) with subsequent thermal desorption ensures highly sensitive detection of VOCs and thus gives the most detailed information about the composition of cell culture headspace.

Interestingly, the results of our experiments showed that the majority of detected volatile substances which are increased compared to medium control are saturated and unsaturated hydrocarbons. However, among the detected hydrocarbons, a statistically significant increase from cells compared to medium control could only be found for 2-methylpentane. Amongst the increased substances, 1-propanol, 2-ethyl-1-hexanol and 2-butanone are more polar compounds, but a significant increase in concentration of polar compounds was only found for 2-ethyl-1-hexanol. However, the results of not significantly increased compounds should not be neglected, since in the group of Phillips *et al*. it was found that several alkanes and branched hydrocarbons were increased in the breath of lung cancer patients compared to that of healthy persons (6). We observed that 2-methylpentane, 3-methylpentane, 2-butanone and 1-propanol were increased in some lung cancer patients and *not* in healthy controls (results not shown). In summary, these results give evidence that various compounds such as hydrocarbons (saturated and unsaturated), ketones and alcohols can be increased in exhaled breath *and* in the headspace of lung cancer cell lines and therefore are compounds which should be taken into account as possible tumor markers.

Another interesting fact is that the NCI-H2087 cell line changed its behavior with respect to the release of VOCs with increasing passage number. 2-Ethyl-1-hexanol, for example, could be detected in the experiments shown here with high peak area. Additional preliminary experiments, nevertheless, showed that with higher passage number of the cells, this compound could no longer be detected. Such a change in behavior was rather specific for 2-ethyl-1-hexanol and could not be seen for other compounds. For instance, the concentrations of other compounds such as 2,4-dimethylheptane and 4-methyloctane did not decrease in the headspace of NCI-H2087 cells with higher passage number.

Our experience shows that the actual concentration differences between the already high medium controls and the cells with regard to these compounds are rather low. A notable exception is 2-ethyl-1-hexanol where only a very low background peak was found. Moreover, although the release of compounds can be confirmed, background peak areas are considerably different in independent experiments which results in high standard deviation and therefore in *p*-values greater than 0.05. Even though a qualitative difference between medium controls and cells can often be found, the quantitative difference varies considerably.

The compounds already present in the headspace of culture medium may, in particular, originate from FCS added to the culture medium. Thus, to reduce this background, one option would be to use medium without serum or to reduce serum to low concentrations. However, preliminary experiments indicated a loss of VOC release under low serum cell culture conditions, possibly due to different metabolism of the cell line used, thus to achieve a lower background level FCS was only reduced to 5% .

The metabolic origin of the detected substances so far remains speculative. A clinical study showed that exhalation of saturated and branched alkanes was increased in persons with increased lipid peroxidation, including smokers and patients infected with human immunodeficiency virus or suffering from inflammatory bowel disease (14). This fact would fit our results where particularly similar compounds were detected *in vitro* but no statistical significance except for 2-methylpentane was found for the investigated cell cultures. However, in literature, fatty acid synthase is described as an enzyme that is activated in cancer (23-25). Thus, lipid peroxidation would not be expected to be a typical metabolic feature of cancer cells *in vivo* and therefore nor *in vitro*.

The results for acetaldehyde were surprising since Smith *et al*. found increased concentrations of acetaldehyde in two lung cancer cell lines (26). Thus, the results of this work are strongly contradictory to our investigations. One reason for this discrepancy may lie in the different cell lines analyzed. In literature, acetaldehyde is described as the first metabolic product of alcohol metabolism *in vivo* (27) and as a carcinogen. However, in the given culture conditions, this alcohol metabolic reaction is negligible since ethanol is not an ingredient of culture media.

Other interesting and surprising facts are that there are several other compounds that seem to be consumed by the cells, such as 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and the ester butyl acetate. In addition, hexanal decreased in all three measurements but because of the high background level and its variations, statistical significance was not confirmed. As for acetaldehyde, the mechanism of consumption of aldehydes by the cells needs to be elucidated. However, the degradation or consumption of aldehydes in the tested cell line may be due to increased activity of aldehyde dehydrogenases. For instance, aldehyde dehydrogenase 1 was found to be a marker of breast cancer cells (28) and was found to be increased in the A549 lung cancer cell line (29). Moreover, in work of Patel *et al*. (30), significantly higher expression levels of aldehyde dehydrogenase 1A1 and aldehyde dehydrogenase 3A1 were detected in squamous cell cancer, adenocarcinoma and small cell lung cancer. They showed that atypical pneumocytes demonstrated

significantly higher levels of expression of aldehyde dehydrogenase 1A1 and aldehyde dehydrogenase 3A1 than normal pneumocytes (a normal counterpart of adenocarcinoma), which is suggestive of up-regulation during malignant transformation to adenocarcinoma. It was also found that non-small cell lung cancer expressed very high levels of aldehyde dehydrogenase 1A1 and aldehyde dehydrogenase 3A1 in comparison with small cell lung cancer. Thus, elevated expression of both enzymes may be associated with malignant transformation to adenocarcinoma. These data would point to a putative metabolic mechanism of degradation of aldehydes which was observed for the NCI-H2087 adenocarcinoma cell line.

In summary, this work is a first step towards a better understanding of the release of VOCs from cancer cells. We found that at least two substances, 2-methylpentane and 2-ethyl-1-hexanol, can be released from the NCI-H2087 lung cancer cell line. Moreover, several other substances, especially alkanes and hydrocarbons, are possibly released from this cell line. It was also clearly shown that these cells consume several types of aldehydes and butyl acetate. Thus, these investigations have given some indication as to which substances are candidates for the most important goal of our project, which is the noninvasive early detection of lung cancer by breath gas analysis.

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