The volatilomic signature of human gastric cancer cell lines (HGC-27 and CLS-145)

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Introduction

Analysis of **volatile organic compounds (VOCs)** released by human body provides an emerging approach for cancer screening. Human volatiles can have a systemic origin or stem from exogenous sources such as environmental exposure, diet or microbiota activity. These compounds form specific biochemical signatures, which can be altered by abnormal processes occurring in the organism, including cancer. The alterations can origin from changes in enzyme activity, modifications of proteins, or activation of genes. The detection of these changes via analysis of VOCs emitted by breath, skin and other bodily fluids provides a unique opportunity to screen or monitor various diseases including cancer.

The main goal of this study was to determine the volatile metabolomic signatures of two selected gastric cancer cell lines: **HGC-27** (Human Gastric Carcinoma) and **CLS-145** (Human Stomach Adenocarcinoma) and one normal line **HSEC** (Human Stomach Epithelial Cells) and detect possible differences between their fingerprints. More specifically, this involved determination of volatiles that are produced and used through metabolic processes of the cells and identifying changes in VOCs production that are caused by the cancer.

Cell lines

The **CLS-145** cell line was established from fragments of a gastric papillary adenocarcinoma of pars cardiac resected from a female therapy-naive patient (European).

The **HGC 27** cell line was derived from a gastric cancer patient with a histologically diagnosed undifferentiated carcinoma.

Non-carcinoma Human Stomach Epithelial Cells (**HSEC**) were isolated from normal human stomach tissue and served as a control.

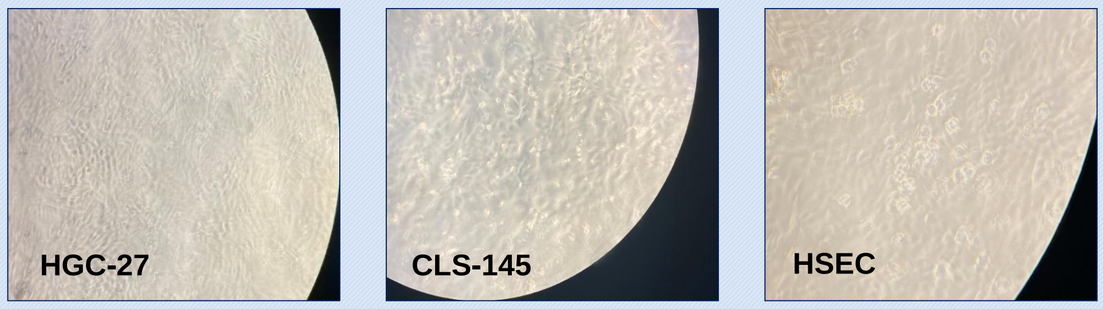


Figure 1. Microscopic pictures of tested cell lines

Results

Amongst the volatiles detected, **twenty seven** showed differences in their headspace concentrations compared to those above the cultivation medium only.

Twelve species were found to be consumed and ten were produced by all cell lines. Of **consumed volatiles** there were *eight aldehydes* (2- methylpropanal, 2-methyl-2-propenal, 2-methylbutanal, 3-methylbutanal, hexanal, heptanal, nonanal and benzaldehyde***),*** *three heterocyclic compounds* (2-methyl-furan, 2-ethyl-furan and 2-pentyl-furan) and *one sulphur containing compound* (dimethyl disulphide). The **produced VOCs** embraced *seven ketones* (2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone, 2-tridecanone, 2-pentadecanone, 2-heptadecanone), *three esters* (ethyl acetate, ethyl propanoate and ethyl 2-methylbutyrate), *three alcohols* (2-methyl-1-butanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol), *one aromatic compound* (toluene) and *one sulphur containing compound* (2-Methyl-5-(methylthio) furan).

HGC-27 cancer cell lines were found to have significantly altered metabolism in comparison to normal gastric cells and **CLS-145** cell line. This was manifested by the increased production of methyl ketones containing an odd number of carbons. **There were also three ketones produced exclusively by this line (2-undecanone, 2-tridecanone and 2-heptadecanone)**. Surprisingly, **HGC-27** produced also toluene. Another interesting feature of the HGC-27 volatile footprint is the lowered production of alcohols and esters.

The **CLS-145** cells exhibited less pronounced changes in their chemical signature. Their volatile footprint is characterised by the upregulated production of esters and 2-ethyl-hexanol and downregulated production of other alcohols.

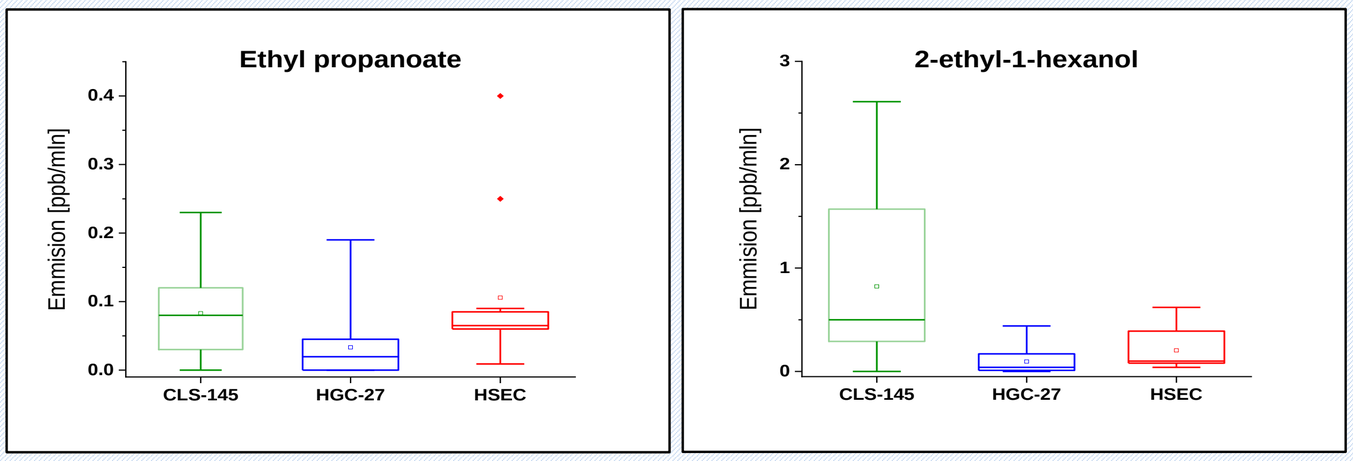
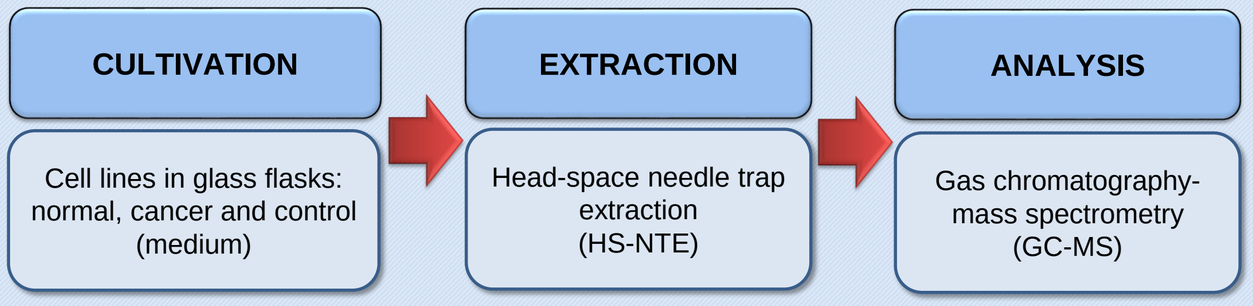


Figure 3. Normalized concentrations [ppb/mln] of ethyl propanoate, 2-ethyl-1-hexanol, 2-ethyl-2-methylbutanoic acid, 2-tridecanone and 2-heptanone in the HS of tested cell lines

Methods: HS-NTE-GCMS analysis

In this study, **headspace needle trap extraction** (HS-NTE), as the pre-concentration method, and **gas chromatography with mass spectrometric detection** (GC-MS) have been applied to respectively capture and analyse the headspace above the cultivating medium and cells in the cultivating medium.



All cell lines were cultivated in glass flasks of 1L closed with a Teflon plug equipped with a rubber septum enabling the insertion of the needle trap devices into the headspace of the bottle (Figure 2).

Altogether 12 sets of cultures containing each three cell cultures and one medium without cells were prepared. The mean number of cells was 22×10^6, 28×10^6 and 12×10^6 for HSEC, HGC-27 and CLS-145 respectively.

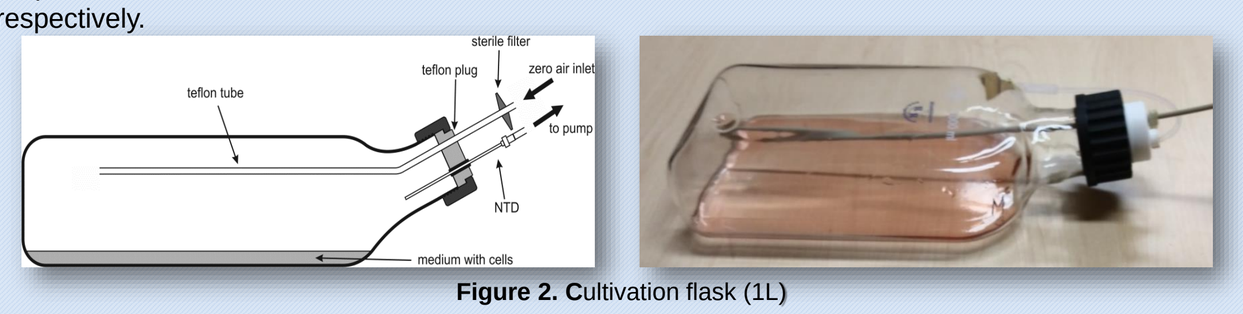


Figure 2. Cultivation flask (1L)

Table 1. Comparison of the emmision of volatile organic compounds between the tested cells lines (↑ - upregulation, ↓ - downregulation)

	Alcohols	Ketones	Ethyl acetate	Ethyl propanoate	2-methyl-5-(methylthio)furan	Toluene
HGC-27 vs CLS-145	↓	↑	↓	↓	↑	-
HGC-27 vs HSEC	↓	↑	↓	↓	↑	-
CLS-145 vs HSEC	↓	-	↑	-	-	-

Table 2. Consumption and emission of VOCs by HGC-27, CLS-145 and HSEC cells related to the medium. Outcome of a Wilcoxon signed rank test. (n.s.–not significant)

	VOC	HGC-27 vs Medium p-value	CLS-145 vs Medium p-value	HSEC vs Medium p-value
UPTAKE	**Aldehydes**			
	2-Propenal, 2-methyl-	4.9×10^{-4}	9.8×10^{-4}	4.9×10^{-4}
	Heptanal	3.4×10^{-3}	1.9×10^{-3}	9.8×10^{-4}
	Nonanal	3.7×10^{-2}	5.9×10^{-3}	7.8×10^{-3}
	Benzaldehyde	2.4×10^{-3}	9.8×10^{-4}	4.9×10^{-4}
	Heterocyclic compounds			
	Furan, 2-pentyl-	1.6×10^{-2}	1.9×10^{-3}	4.9×10^{-4}
	Furan, 2-ethyl-	4.9×10^{-4}	9.8×10^{-4}	4.9×10^{-4}
	Sulphur containing compound			
	DMDS	7.8×10^{-3}	1.6×10^{-2}	n.s.
RELEASE	**Esters**			
	Ethyl propanoate	n.s.	1.9×10^{-2}	4.9×10^{-4}
	Ethyl 2-methylbutyrate	4.9×10^{-4}	9.8×10^{-4}	4.9×10^{-4}
	Aromatic compound			
	Toluene	2.4×10^{-3}	n.s.	n.s.
	Alcohols			
	1-Butanol, 3-methyl-	2.4×10^{-3}	2.9×10^{-3}	4.9×10^{-4}
	1-Hexanol, 2-ethyl-	n.s.	4.9×10^{-3}	1.6×10^{-2}
	Heterocyclic compounds			
	2-Methyl-5-(methylthio)furan	4.9×10^{-4}	9.8×10^{-4}	4.9×10^{-4}
	Ketones			
	2-Undecanone	4.9×10^{-4}	n.s.	n.s.
	2-Tridecanone	4.9×10^{-4}	n.s.	n.s.
	2-Pentadecanone	4.9×10^{-4}	1.9×10^{-2}	1.5×10^{-3}
	2-Heptadecanone	4.9×10^{-4}	n.s.	n.s.

Conclusions

a) the results obtained during the studies provide evidence that gastric cancer alters the VOCs profiles of the cell lines under study
b) twelve VOCS were found to be produced and fifteen metabolized by the cells of interest
c) qualitative and quantitative differences in the cells volatilomic footprints were detected.
d) the observed volatiles might have a value as biomarkers for gastric cancer diagnosis (via e.g. breath, or urine analysis) based on sensitive mobile platforms (e.g. enose)

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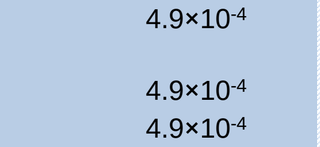

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