Analysis of Small Cell Lung Cancer specific Volatile Organic Compounds in Hypoxic Conditions

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Background

Lung cancer is the leading cause of cancer-related deaths globally, where 84% of cases are diagnosed late, when treatments are no longer effective [1]. Limitations in current diagnostic tools have resulted in dedicated research identifying volatile organic compounds (VOCs) specific to cancer [2]. Due to altered biochemical pathways, it is hypothesized that different cancer cells have unique VOC expression [1]. This study utilizes recent research by examining VOCs specific to small cell lung cancer in normal and hypoxic conditions. The VOCs were initially collected from the headspace of the Biodome (a custom glass culture dish interconnected to a gas flow system) using a specialized sorbent carbon material. The samples were then run through a comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry and the VOC relative abundances were analyzed. The results revealed unique VOC patterns validated by metabolic pathways. These VOC patterns can then be utilized by oncologists to help them characterize and stage the type of lung cancer in a noninvasive way.

Experimental Methods

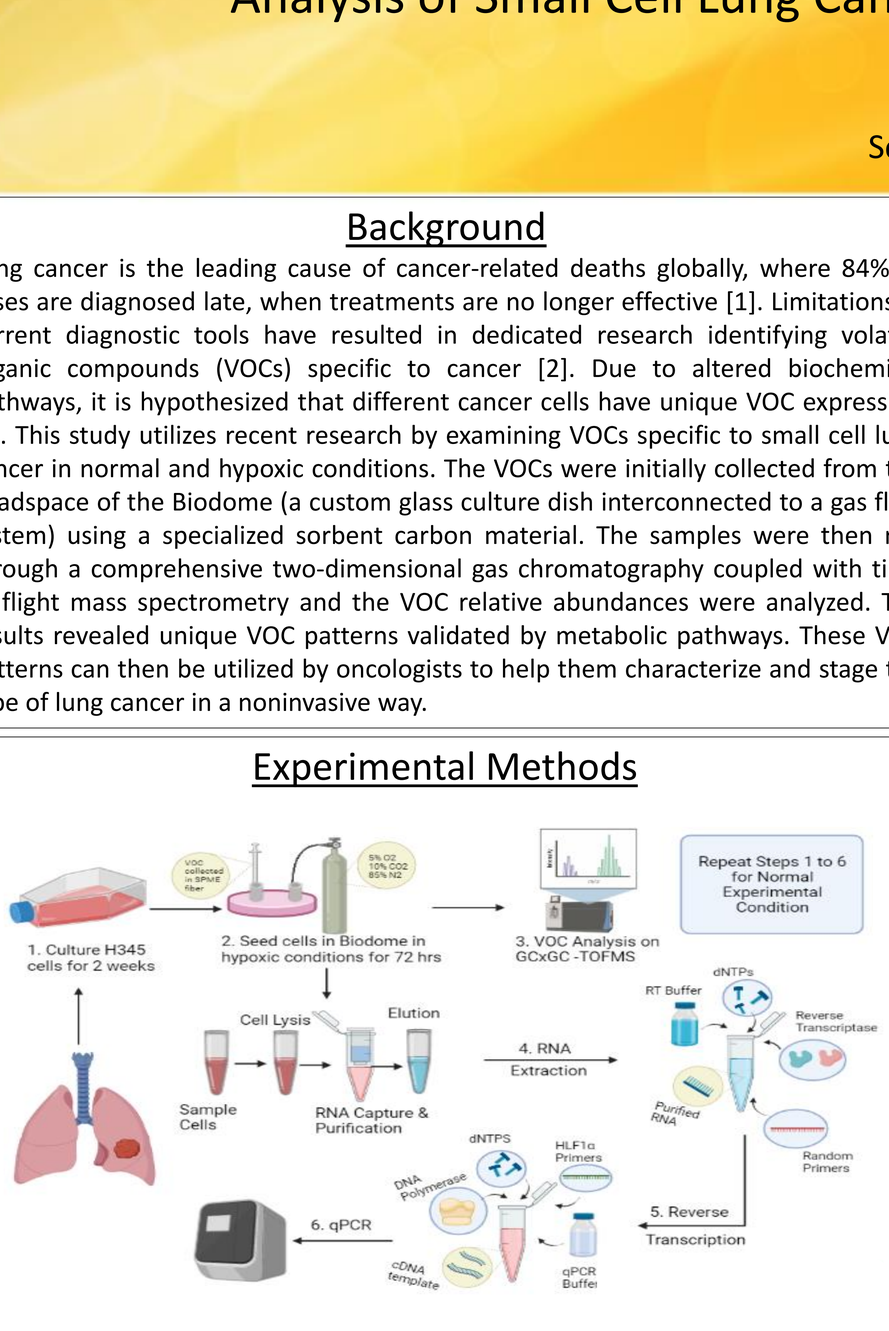


Figure 1. A schematic of a step by step experimental procedure used to measure VOCs specific to H345 cells in hypoxic and normoxic conditions over a period of 72 hrs. The procedure also involves the validation of the hypoxic lung cancer model by measuring the expression of HIF1α gene in the cells through qPCR.

Research Goals

The goal of the research project is to identify VOCs specific to small cell lung cancer in the hypoxic headspace of in vitro cell cultures.

Results

Absorption Spectra of RNA and cDNA in Hypoxic and Normoxic H345 Cells

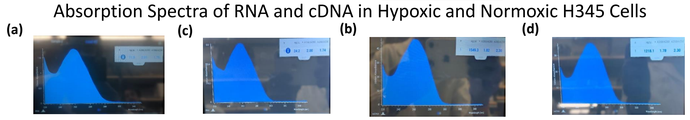
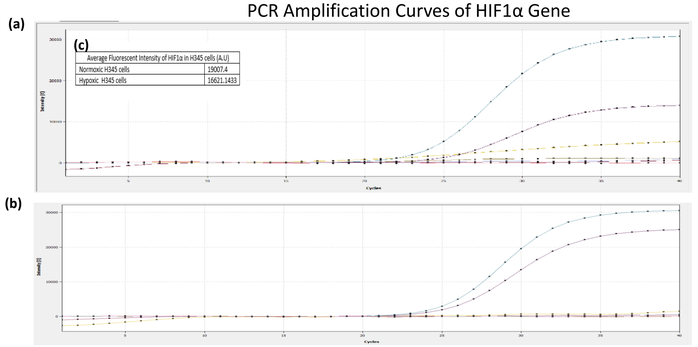


Figure 3. Absorbance Spectrum of the RNA in normoxic (**a**) and hypoxic (**b**) conditions, along with cDNA in normoxic (**c**) and hypoxic (**d**) conditions at ratios of A260/280.

PCR Amplification Curves of HIF1α Gene



H345 Cell Images

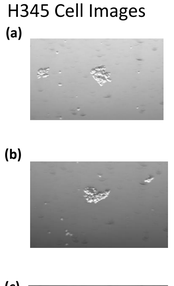


Figure 2. Images of H345 cells taken after (**a**) 24, (b) 48, and (**c**) 72 hrs. in normoxic conditions

Figure 4. PCR amplification curve of the HIF1α gene for (**a**) normoxic and (b) hypoxic H345 cells. (**c**) Table illustrating a higher average fluorescent intensity of HIF1α gene for the hypoxic H345 cells compared to normoxic H345 cells at the end of cycle 40 of qPCR.

Mass Spectra of VOCs Specific to Normoxic and Hypoxic H345 Cells

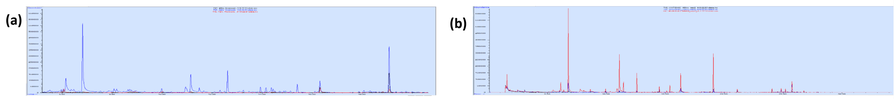


Figure 5. Mass chromatogram of VOCs specific to (**a**) normoxic and (**b**) hypoxic H345 cells within the 24, 48 and 72 hrs. time periods.

Box, Line and Bar plots of abundant VOCs in Normoxic and Hypoxic H345 cells

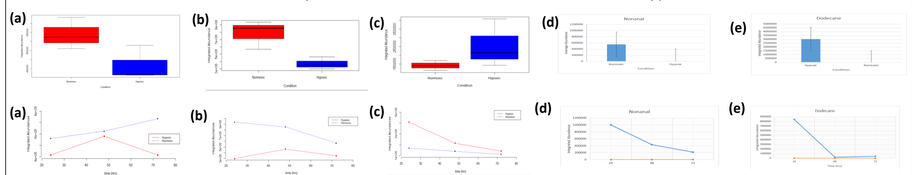


Figure 6. Box and bar plots illustrating chemical abundance of (a) Cyclotetrasiloxane and octamethyl- (b) Cyclotetrasiloxane and hexamethyl– (c) Tetradecane (d) Nonanal and (e) Dodecane in normoxic and hypoxic H345 cells. Line plots illustrating the shift of abundances of (a) Cyclotetrasiloxane and octamethyl- (b) Cyclotetrasiloxane and hexamethyl – (c) Tetradecane (d) Nonanal and (e) Dodecane in normoxic and hypoxic H345 cells over 72 hrs.

Obstacles

One of the hypoxic sample runs had to be repeated due to a technical error in the insertion of the SPME fiber into the mass spectrometer which led to the loss of the VOC collection data. The solution volumes for the reverse transcription were very low which made it difficult to prepare the cDNA for the qPCR step.

Conclusion

Through the data analysis of the metabolites measured across the 72 hrs. time period, unique VOC patterns have been identified for both hypoxic and normoxic H345 cells. There was an increased expression of VOCs such as cyclotetrasiloxane - hexamethyl, cyclotetrasiloxane - octamethyl, and nonanal in normoxic H345 cells. On the other hand, VOCs such as Tetradecane and Dodecane were found more abundant in hypoxic H345 cells. These VOCs can then be used to detect small cell lung cancer and stage the disease as the increased abundance of normoxic VOCs would indicate an early stage of SCLC while hypoxic VOCs are associated to a later stage of the disease. Future work will include examining the VOCs when chemotherapeutics are induced to the cells to identify the cancer treatment response in non invasive way.

Acknowledgments

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References

[1] Jia, Zhunan, et al. “Detection of Lung Cancer: Concomitant Volatile Organic Compounds and Metabolomic Profiling of Six Cancer Cell Lines of Different Histological Origins.” ACS Omega, American Chemical Society, 31 May 2018, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6044508/.

[2] Lazris, Andy, and Alan R. Roth. “Lung Cancer Screening: Pros and Cons.” American Family Physician, 15 June 2019, https://www.aafp.org/afp/2019/0615/p740.html.

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