Mass spectrometry imaging of the metabolic landscape of human papillomavirus-associated versus carcinogen-driven head and neck squamous cell carcinoma.

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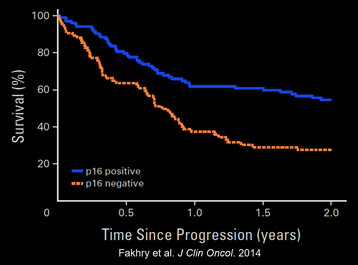
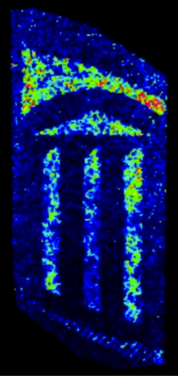
Background

Mass spectrometry imaging of head and neck squamous cell carcinoma has not been performed prior to this study. Using this state-of-the art technique, we are now able to elucidate the spatial enrichment of metabolites in order to define cell-specific metabolism which can be leveraged to enhance the anti-tumor immune response. We chose to leverage MALDI-MSI to interrogate a set of head and neck squamous cell carcinomas, a lethal, disfiguring form of cancer with limited treatment options including highly morbid surgery, cytotoxic chemoradiation, or immunotherapy. We sought to compare the metabolic landscapes of human papillomavirus (HPV)-related head and neck squamous cell carcinoma (HNSCC) vs carcinogen-driven HNSCC as the two most common etiologies of head and neck cancer. From a public health perspective, HPV-related head and neck squamous cell carcinoma (HNSCC) is increasing in incidence while carcinogen-driven HNSCC is decreasing.[1] HPV-related HNSCC is expected to continue rising in incidence until at least 2060.[2] In addition, HPV-related HNSC incidence is rising, and prognosis is poor for recurrent cancer.[3] Understanding the metabolism of these tumors will allow us to design metabolic therapies to diminish cell proliferation and increase the anti-tumor immune response. This study will use MALDI-MSI to study the metabolic milieu of HPV-associated versus carcinogen-driven head and neck squamous cell carcinomas.



Fakhry et al. *J Clin Oncol*. 2014

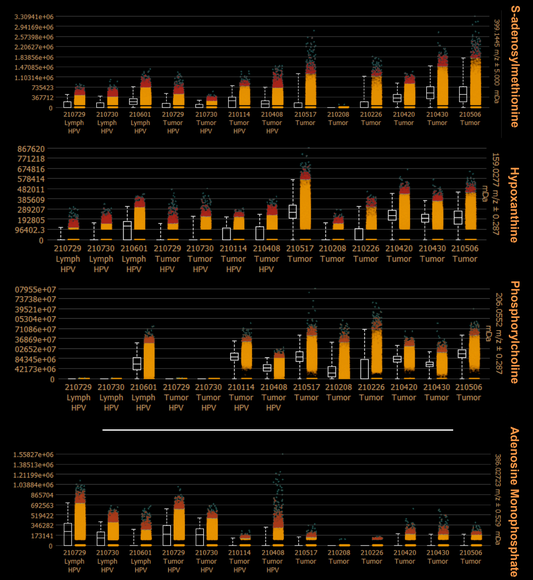
Methods



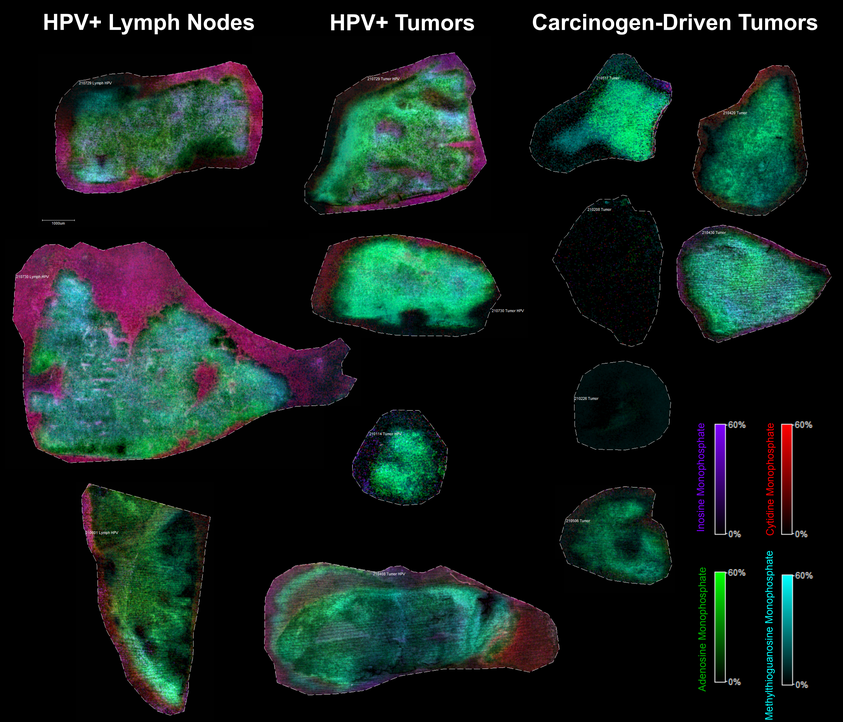
Human head and neck squamous cell carcinomas (HNSCs) were surgically removed and slow frozen over liquid nitrogen. Samples were then embedded in gelatin and stored at -80 degrees Celsius until cryosectioned into 15-micron sections. All sections were thaw mounted onto ITO-coated glass slides. Slides were then coated in 15 mg/mL alpha-cyano-4-hydroxy-cinnamic acid (CHCA) matrix using an HTX-Imaging M3 TM-Sprayer (6 passes, 75ºC. Sections were subjected to MALDI-MSI analysis using a 12T Bruker Solarix FT-ICR at 25-micron raster width. Instrument resolution was set to 128K to maintain manageable file sizes and run times. MALDI imaging data was visualized in flexImaging. Statistical analysis of imaging data was conducted using SCiLS Lab v2016b. All data was uploaded to Metaspace for analyte annotation.

Results & Conclusion

A total of seven HPV-associated (three metastatic lymph nodes and four primary tumors) and six carcinogen-driven (primary tumors) HNSC specimens were analyzed using MALDI-MSI. Discriminative analysis between HPV-associated HNSC and carcinogen-driven HNSC revealed that HPV-associated tumors were significantly enriched in **glutamine**, **inosine monophosphate**, **spermidine**, **spermine**, **xanthine**, and **indole-3-carboxyaldehyde**. Metabolites significantly enriched in carcinogen-driven HNSC relative to HPV-associated HNSC were **s-adenosylmethionine**, **hypoxanthine**, and **phosphorylcholine**. **Adenosine monophosphate** was enriched in HPV-associated metastatic lymph nodes compared to all other samples. This is particularly interesting, as several studies have reported that the conversion of extracellular adenosine monophosphate to adenosine, via the CD73 ectonuclease, restricts lymphocyte migration into the lymph nodes, contributing to immunosupression.[4,5] In addition to adenosine monophosphate, several other nucleotides were also more intense in HPV-driven tumors, possibly suggesting an increase in nucleotide synthesis, contributing to virulence.[6]



Overall, several candidate metabolites were differentially enriched in HPV-associated primary tumors or lymph nodes compared to carcinogen-driven primary HNSCs.



Future Directions

In ongoing analyses, we are staining for CD8+ T cell and macrophage markers to correlate metabolite enrichment with immune cell infiltration to evaluate for evidence of immune cell exclusion or enrichment. Correlations between CD8+ staining and MALDI imaging data will give insight into possible metabolic pathways of interest.

These MSI studies further develop methodology previous used and that is in the process of being applied to studies of lymph node after ionizing radiation. Our previous MSI of mesenteric lymph node after exposure to ionizing radiation showed depletion of lymphoid cell populations and alteration of lipid abundance and localization which were accompanied by proteomic changes consistent with dysregulation of both lipids and metabolites relevant to immune response and inflammation [7]. This work develops the capabilities to image metabolites which will allow a more thorough characterization of immune relevant species in animal models used for drug development of medical countermeasures.

Sources & Funding

1. Kulkarni PR, Rani H, Vimalambike MG, Ravishankar S. Asian Pac J Cancer Prev. 2013; 14(9):5101-5.
2. Stephen JK, Divine G, Chen KM, Chitale D, Havard S, Worsham MJ. Cancer Clin Oncol. 2013; 2(1):51-61.
3. C. Fakhry *et al.* J Clin Oncol. 2014; 32(30):3365-73.
4. Prabhu SR, Wilson DF. Aust Dent J. 2013 Mar; 58(1):2-10.
5. Takedachi M, *et al.* J Immunol. 2008 May 1;180(9):6288-96.
6. Ariav Y, et al. Scince Advances. 2021; 7(21).
7. Muller L. et al. Health Phys. 2021; 121 (4):372-383

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