Evaluating Chemoradiation Resistance of Head and Neck Cancer Via Multiscale Analysis of Tumor Heterogeneity and Cell Topology

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ABSTRACT

Purpose
Understanding why tumors resist chemoradiation remains a major barrier to improving outcomes in head and neck cancer. We developed a multiscale framework linking tumor architecture, cellular network topology, and immune context to therapeutic response, treating tumors as spatially organized networks rather than homogeneous masses. This approach aims to identify topology-driven biomarkers of resistance and survival, providing interpretable insights into tumor behavior.

Methods
Phase 1: mouse oral carcinoma cell lines MOC1 (n=42), MOC2 (n=36), and HPV-positive MLM1 (n=39) were orthotopically implanted in the buccal mucosa. Chemoradiation (cisplatin 5 mg/kg and 8 Gy on days 0 and 7) was initiated at 50mm³, with CT imaging on day 14 and follow-up to endpoint. Radiomic features were extracted and modeled using LASSO-Cox regression. Phase 2: MOC2 tumors were treated once and harvested at days 1, 3, or 7 post-treatment for digital pathology. In Phase 3: MOC2 tumors with or without Ccr2 knockout (n=14) were followed to endpoint to assess immune heterogeneity. Pathomic features capturing tumor topology were computed at local (10.5μm) and global (15.75μm) scales.

Results
Phase 1 identified five significant radiomic predictors reflecting tissue heterogeneity. Phase 2 showed slow-growing tumors had higher average degree and k-core values, indicating more organized networks early after treatment. Phase 3 demonstrated fast-growing tumors had increased local heterogeneity, clustering deviation, and correlation variance, whereas lower eigenvector centrality and correlation mean were linked to improved survival. Highly connected k-core structures characterized aggressive tumors, while increased high-betweenness cells and lymphocytic density predicted better outcomes. Across scales, architectural heterogeneity correlated with therapeutic and immune vulnerability, and topological metrics evolved over time, reflecting dynamic tumor network remodeling.

Conclusion
Chemoradiation-resistant tumors exhibit coordinated multiscale disruptions in topology and architecture. Topology-informed radiopathomic modeling provides an interpretable framework for survival prediction and treatment stratification, highlighting spatial organization as a determinant of tumor behavior.

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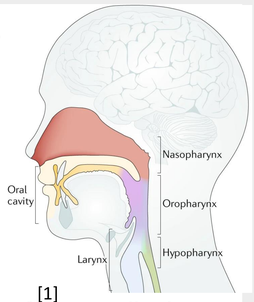
INTRODUCTION

- Head and neck squamous cell carcinoma (HNSCC) can originate in several anatomical locations, including the oral cavity, oropharynx, hypopharynx, and larynx [1]
- Radiomics uses computational methods to extract quantitative features from medical images, enabling objective characterization of tumor heterogeneity and disease phenotype [2]
- Pathomics applies computational analysis to digital pathology images to quantify cellular and tissue architecture, providing objective measures of immune infiltration and tumor microenvironment heterogeneity that may serve as prognostic and predictive biomarkers [3]
- This study is motivated by the need to understand and predict heterogeneous treatment responses in HNSCC by linking macroscopic radiomic features with microscopic pathomic architecture and tumor microenvironmental influences within a unified multi-scale framework



[1]

METHODS

Phase 1 (macroscopic texture): Three cell lines were injected into mouse buccal mucosa: **(1)** MOC1(300,000 cells/mouse, n=42) and **(2)** MOC2 (30,000cells/mouse, n=36), which are mouse oral carcinoma (MOC) cell lines developed in C57BL/6 mice through carcinogen exposure, and **(3)** MLM1 (300,000cells/mouse, n=39), which is an HPV-positive and immune-competent cell line expressing HPV16 E6 and E7 proteins. Treatment was initiated when tumors reached 50mm^3 using caliper measurements and cylindrical approximation on day 0 and 7 with 5mg/kg cisplatin and 8Gy radiation, and CT images were acquired on day 14 on a small animal μCT scanner. Mice were then followed until endpoint (tumors reaching 1cm in any direction). Radiomic features were then extracted from the CT images for multivariate modeling.

Phase 2 (microscopic cell dynamics): Mice were induced with 30,000 MOC2 cells orthotopically implanted into the buccal mucosa and treated with 5mg/kg cisplatin and 8Gy radiation once. They were then sacrificed at 1 (n=6), 3(n=5), or 7(n=5) days post-treatment and tumor tissue samples were taken for digital pathology. Pathomic features capturing tumor cell topology were analyzed at two edge distances (10.5um, cell-cell interactions, and 15.75um, global tissue architecture).

Phase 3 (macrophage knockout): 14 mice were induced (MOC2) and treated, where 7 mice had their CCR2 gene knocked out (-/-) to introduce macrophage density diversity. 7 mice reached treatment threshold on day 14, and 7 on day 31. Mice were followed until their tumors reached 1 cm in any dimension, at which point tumors tissue samples were taken for digital pathology. Pathomic features capturing tumor cell topology were analyzed at two edge distances (10.5um and 15.75um)

RESULTS

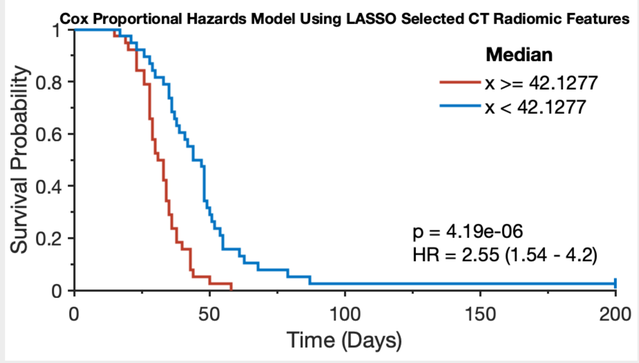


Figure 1. LASSO selected CT radiomic features provided prognostic information when applied to a Cox Proportional Hazards model.

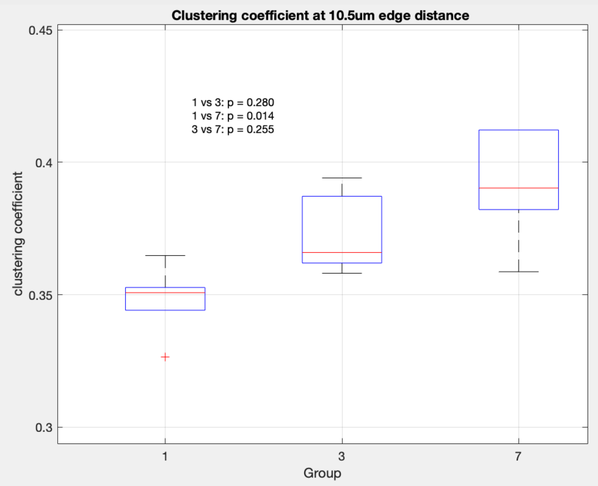


Figure 2. Six tumor topology pathomic features were significantly different between day 1 and day 7, including the clustering coefficient, which increased as time post radiation increased.

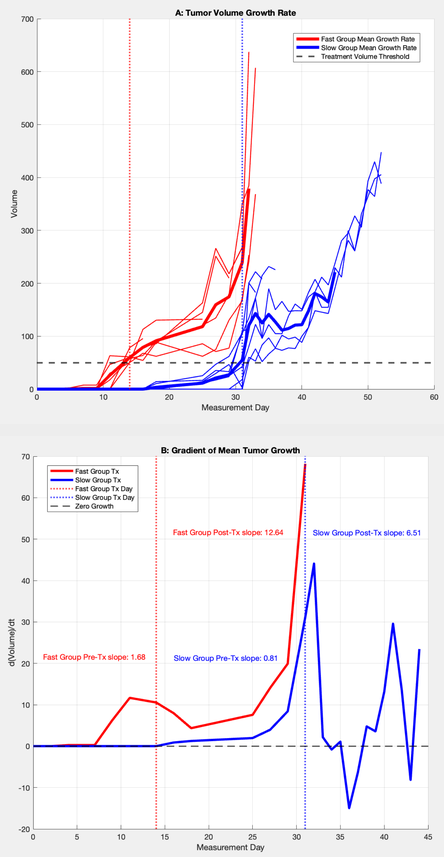


Figure 3a and 3b: Mice that reached treatment threshold size on day 14 responded less to chemoradiation than the slower growing cohort that reached treatment threshold on day 31.

RESULTS

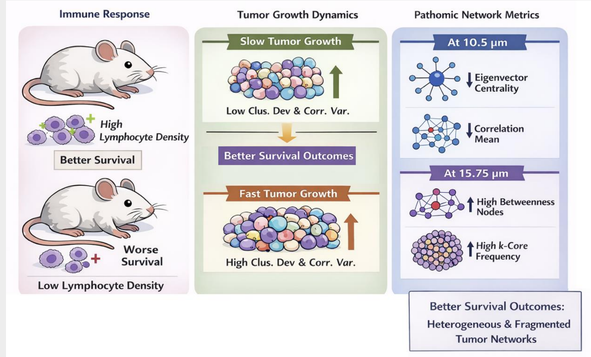


Figure 4. Mice with increased survival shared pathomic features, such as decreased eigenvector centrality and increased k-core frequency.

DISCUSSION

- These findings support the value of integrating radiomic and pathomic approaches to better capture tumor heterogeneity across scales, as imaging-derived signatures and tissue-level topology both showed associations with clinically relevant outcomes
- The prognostic performance of LASSO-selected radiomic features highlights the potential of CT-based quantitative imaging as a noninvasive tool for risk stratification in HNSCC models
- The observed temporal changes in tumor topology following radiotherapy suggest that treatment induces measurable reorganization of the tumor microenvironment, which may not be fully captured by macroscopic imaging alone.

CONCLUSIONS

Together, these results indicate that growth kinetics, tissue architecture, and network-based cellular organization can potentially provide prognostic information regarding treatment response and survival, supporting the development of multi-scale predictive frameworks for therapeutic stratification.

CITATIONS

[1] Cramer, J. D., Burtness, B., Le, Q. T., & Ferris, R. L. (2019). The changing therapeutic landscape of head and neck cancer. *Nature reviews. Clinical oncology*, *16*(11), 669–683. https://doi.org/10.1038/s41571-019-0227-z
[2] Lafata, K. J., Wang, Y., Konkel, B., Yin, F. F., & Bashir, M. R. (2022). Radiomics: a primer on high-throughput image phenotyping. *Abdominal radiology (New York)*, *47*(9), 2986–3002. https://doi.org/10.1007/s00261-021-03254-x
[3] Li, X., Heirman, C. C., Rickard, A. G., Sotolongo, G., Castillo, R., Adanlawo, T., Everitt, J. I., Hodgin, J. B., Watts, T. L., Janowczyk, A., Mowery, Y. M., Barisoni, L., & Lafata, K. J. (2024). Computational staining of CD3/CD20 positive lymphocytes in human tissues with experimental confirmation in a genetically engineered mouse model. *Frontiers in immunology*, *15*, 1451261. https://doi.org/10.3389/fimmu.2024.1451261

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