#171 : A novel single-cell level approach integrating artificial intelligence (AI)-powered histomorphology labeling and spatial transcriptomics enables biomarker identification of treatment-resistance in salivary gland cancer (SGC)

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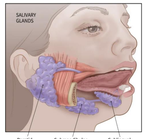
Background

- Salivary gland cancer (SGC) encompasses several rare tumor types with unmet clinical needs (Geiger, JCO 2021).
  - Accounts for ~10% of all head and neck cancers.
  - Heterogeneous pathological subtypes & varied responses to therapies.
- Detailed characterization of SGC tumor microenvironments (TME) across diverse clinical samples is needed to enhance clinical outcomes.
  - Promising methods:
    - AI-powered pathology image analysis (H&E-AI)
    - Spatial transcriptomics (ST)

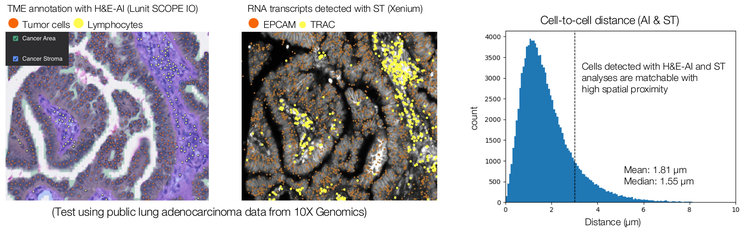


(Image source: American Cancer Society)

Method: integrating H&E-AI and spatial transcriptomic (ST)

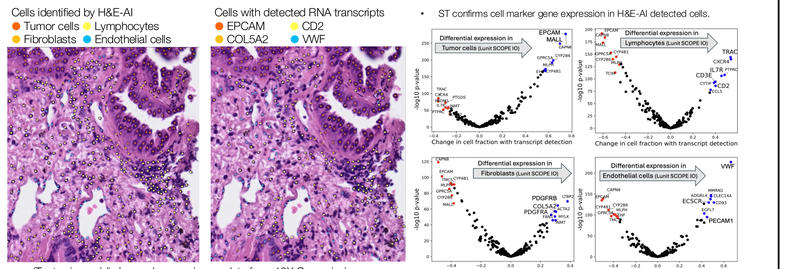
- For comprehensive characterization of SGC, we developed an analysis pipeline to integrate
  - AI-based pathological image analysis (Lunit SCOPE IO)
  - Spatial transcriptomics (10X Genomics Xenium)
- We validated the integration pipeline on various H&E-Xenium data pairs.

Parallel characterization of tumor microenvironment (TME) using H&E-AI and ST



(Test using public lung adenocarcinoma data from 10X Genomics)

Reciprocal validation of H&E-AI and ST through feature registration



(Test using public lung adenocarcinoma data from 10X Genomics)

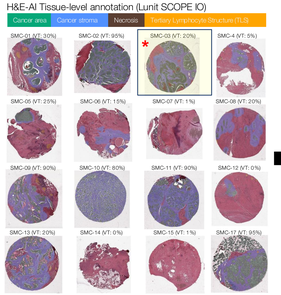
- ST confirms cell marker gene expression in H&E-AI detected cells.

Result 1: Comprehensive characterization of the SGC tumor microenvironment

Cohort: 16 SGC patients pre-treated with neoadjuvant therapy (nivolumab + cytotoxic chemotherapy)

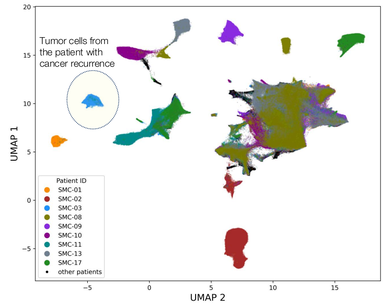
Clinical Outcome:
- Variable proportions of viable tumors (VT) - 0% to 95%
- One patient with cancer recurrence post-surgery (SMC-03)

Data: 16 surgically removed tumor samples
- H&E images
- 10X Xenium spatial transcriptomics data (custom 475 gene panel)



* **Disease recurrence after surgery**

- Total 915,215 cells across the 16 tumors, visualized by gene expression.

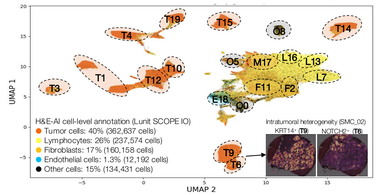


SGC tumor cells display significant heterogeneity in gene expression and cellular morphology

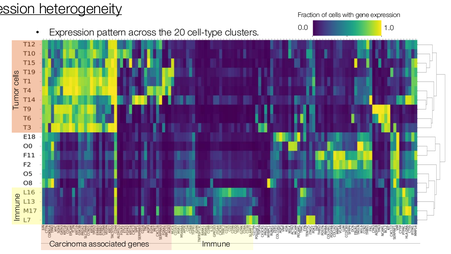
- Observed high gene expression heterogeneity among putative tumor cell subtypes.
- ST based tumor cell detection using reference gene expression profiles was insufficient and required complementary morphological analysis from H&E-AI.
- Integration of H&E-AI and ST identified 10 groups as tumor cell subtypes harboring diverse morphologies.

Gene expression heterogeneity

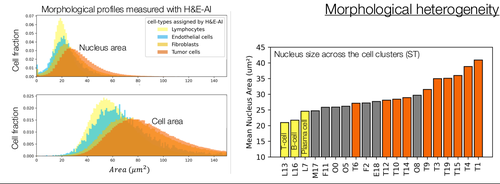
- Identified 20 cell clusters with unique gene expression patterns.
- 10 expression clusters had >70% of the cells labeled as tumor cells by H&E-AI.

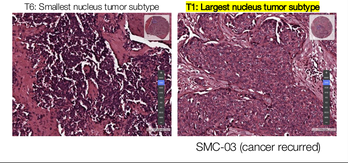


- Expression pattern across the 20 cell-type clusters.



Morphological heterogeneity



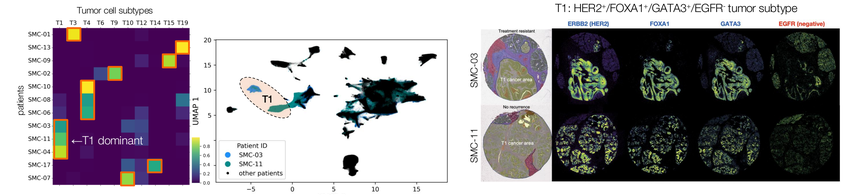


SMC-03 (cancer recurred)

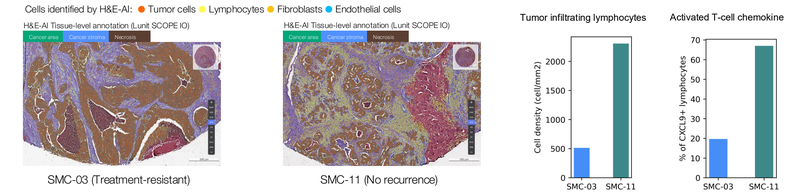
Result 2: Discovering biomarkers for SGC malignancy

Tumor composition profiling identifies patient subgroups with common dominant tumor cell subtypes.

- Three patients with T1 dominant tumor: SMC-11, SMC-04, SMC_03 (recurred).
- Gene expression patterns of SMC-11 and SMC-03 are especially similar (R = 0.92)
- However, only SMC-3 experienced cancer recurrence.

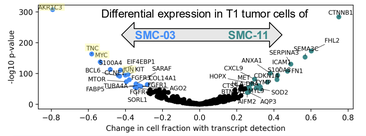


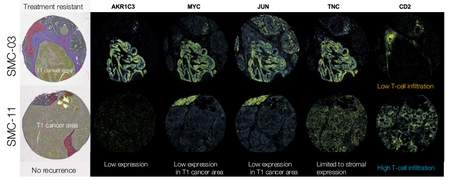
The treatment-resistant tumor (SMC-03) shows weak immune activation relative to the other T1 dominant tumor.



The treatment-resistant tumor (SMC-03) exhibits gene expression associated with immune evasion, metastasis, EMT, and drug resistance in other types of cancers.

- MYC - immune evasion, metastasis (Dhanasekara, NRCO 2022)
- TNC - immune evasion, EMT, metastasis (Yilmaz, JCS 2022)
- JUN - EMT, metastasis (Razavi-Mohseni, GR 2024)
- AKR1C3 - drug resistance (Liu, JMC 2020)





Conclusion

- We integrated **H&E-AI analysis** and **spatial transcriptomics (ST)** to provide a synergistic analysis of tumor microenvironments (TME), offering insights beyond what each technology can achieve individually.
- By combining AI-based cell morphology analysis with spatial transcriptomics, we uncovered **potential mechanisms underlying resistance to neoadjuvant chemo-IO treatment** and identified biomarkers that may guide treatment decisions in SGC.
- Limitation: As this is **an ongoing clinical trial** with survival follow-up, clinical findings should be interpreted with caution until more data is available.
- This approach holds promise for discovering novel biomarkers closely related to the underlying mechanisms of treatment response.