Synthesizing Tumor Infiltrating Lymphocyte Patterns with Genomic Measurements for Head and Neck Cancer Survival

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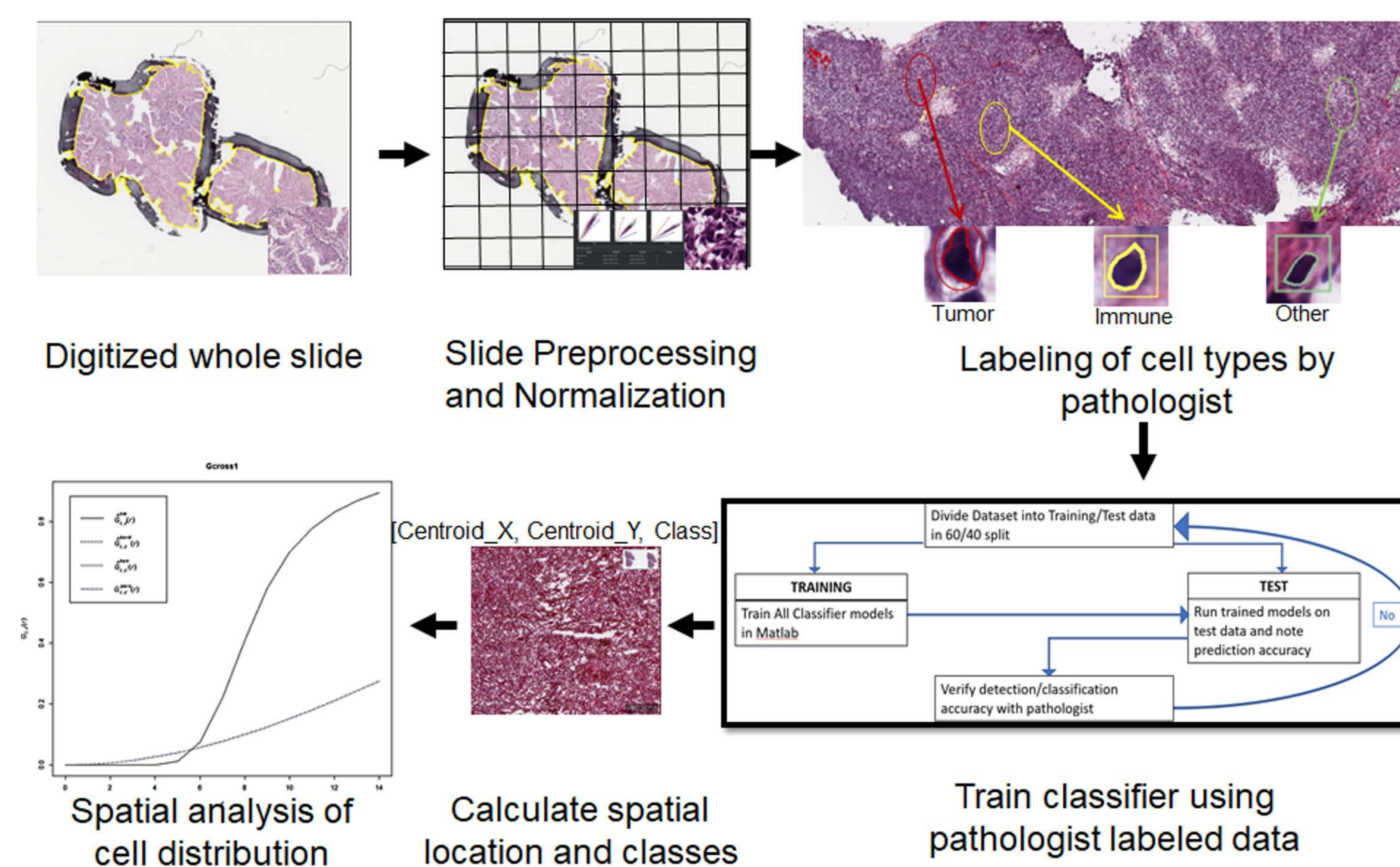
Background

Survival from head and neck squamous cell carcinoma (HNSCC) has remained stubbornly low at ~50%, the relapse rate is high and treatment options are frequently limited and debilitating. For late-stage HNSCC tumors, immunotherapy offers a novel and promising treatment option for ~20-30% of HNSCC cases. Lymphocytes that leave the bloodstream and infiltrate the tumor (tumor infiltrating lymphocytes, or TILs) induce cell death upon activation, and the density of TILs in a tumor has been shown to be an important prognostic indicator for many malignancies, including HNSCC.

Purpose

Our overall goal is to predict the benefit of immunotherapy options for individual HNSCC patients by integrating TIL imaging of readily available H&E slides with clinical information and easily obtained genetic results.

Methods

- Incident HNSCC cases diagnosed from 2008-2014 were recruited through Michigan Medicine Otolaryngology. Subjects with a primary tumor in the oral cavity, oropharynx, larynx, or hypopharynx with an existing specimen were selected for further analysis.
- H&E slides were imaged (40X) through the UM Pathology Slide Scanning Service. We have optimized and completed image analysis for 104 cases (of a total of 500 tumors). Radial distances of 10, 20, 30, and 40 microns were evaluated.

Digitized whole slide → Slide Preprocessing and Normalization → Labeling of cell types by pathologist (Tumor, Immune, Other)

[Centroid_X, Centroid_Y, Class]

Spatial analysis of cell distribution ← Calculate spatial location and classes ← Train classifier using pathologist labeled data

TRAINING — Train All Classifier models in Matlab
TEST — Run trained models on test data and note prediction accuracy
Divide Dataset into Training/Test data in 60/40 split
Verify detection/classification accuracy with pathologist

- G-function scores (split at the median) were compared across clinical factors using ANOVA or Kruskal-Wallis test depending on distribution. Kaplan-Meier curves compared overall and recurrence-free survival between low and high G-function scores.   Cox proportional hazards models were utilized to adjust for clinical covariates. One outlier was excluded from analysis.

Conclusions

- **TIL infiltration, measured by G-function score, is heterogenous across head and neck cancer subsites. Oropharynx cancers appear to have the highest G-function score in our preliminary data, perhaps indicative of an association with HPV+ oropharynx cancer.**
- **G-function score may be predictive of a prognostic advantage in HNSCC but depends on site.**
- **Ongoing analysis include somatic mutation analyses of tumors and G-function score calculation for an additional 396 HNSCCs.**

Results

TABLE 1: Clinical characteristics of preliminary cohort (N (%))	Overall (N=104)
Gender	
Male	65 (62.5)
Age (years)	
Mean	61.4
Smoking Status	
Never	25 (24.0)
Current	45 (43.3)
Former (quit > 12 months)	30 (28.8)
Unknown	4 (3.9)
Drinking Status	
Never	12 (11.5)
Current	70 (67.3)
Former (quit > 12 months)	18 (17.3)
Unknown	4 (3.9)
Disease Site	
Oral Cavity	65 (62.5)
Oropharynx	25 (24.0)
Larynx	11 (10.6)
Hypopharynx	3 (2.9)
Disease Stage - AJCC 7th ed.	
I or II	31 (29.8)
III or IV	73 (70.2)
HPV Status	
Positive	23 (22.1)
Negative	55 (52.9)
Invalid/Missing	26 (25.0)
Treatment Modality	
Surgery alone	34 (32.7)
Surgery + adjuvant radiation	23 (22.1)
Surgery + adjuvant chemorad	17 (16.4)
Radiation alone/Chemo alone	7 (6.7)
Chemorad	17 (16.4)
Palliative/unknown	6 (5.8)
BMI	
Underweight (<18.5)	7 (6.7)
Normal [18.5, 25)	28 (26.9)
Overweight [25, 30)	36 (34.6)
Obese (≥30)	28 (26.9)
Unknown	5 (4.8)
Overall Survival Time (months)	
Mean	32.4

- The majority of the cases were male (62.5%), current smokers (43.3%) and stage III or IV (70.2%).
- Mean age of diagnosis was 61.4 years and mean overall survival time was 32.4 months.
- G-function score (30 microns) differed by HPV-status (0.005) and marginally by disease site (0.09).
- Overall and recurrence free survival was better among higher G-function scores, although the trends were not significant.
- Cox proportional hazard models were stratified by G-function score and adjusted for smoking status, BMI, HPV status. G-function score (30 microns) was stratified at the median.

Overall Survival

Recurrence Free Survival