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Identification of tertiary lymphoid structures from H&E slides using deep learning analysis of nuclear morphology is associated with favorable survival in colorectal cancer patients

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INTRODUCTION

- Mature tertiary lymphoid structures (mTLS) are organized aggregates of immune cells, which support the anti-tumor immune response, through antigen presentation, cytokine production and generation of tumor-specific antibodies. The presence of mTLS in the tumor microenvironment is associated with better prognosis and immunotherapy response across cancer types [1].
- mTLS are defined as prominent B-cell follicles (CD20+) and follicular dendritic cells (CD21+) that are surrounded by T-cells (CD3+). Thus, multiple immunohistochemical stainings or multiplex imaging is typically required for an accurate detection of mTLS, making it challenging to implement as a clinical biomarker.
- In this work we developed a deep learning model that extracts nuclear morphology features to detect mTLS from H&E slides and demonstrated its prognostic role in colorectal cancer (CRC) patients.

METHODS

- A publicly available dataset consisting of 140 tissue cores from 35 CRC patients stained with H&E and 56 protein markers using the CODEX multiplex immunofluorescence (mIF) system was analyzed [2].
- Immune cell aggregates on H&E tissue cores were annotated by expert pathologists as either mTLS or lymphocyte aggregates (LA), based on marker expression from the mIF stain on the same tissue core. mTLS were defined as prominent aggregates containing B-cell follicles (CD20+) and follicular dendritic cells (CD21+) surrounded by T-cells (CD3+), while all other immune cell aggregates were defined as LA.
- HoVer-Net was used to perform nuclear segmentation on cells within the mTLS and LA on the H&E [3]. Nuclear features including eccentricity, solidity, convexity, and nuclear intensity were extracted and the mean and variance of each feature was summarized per annotated region.

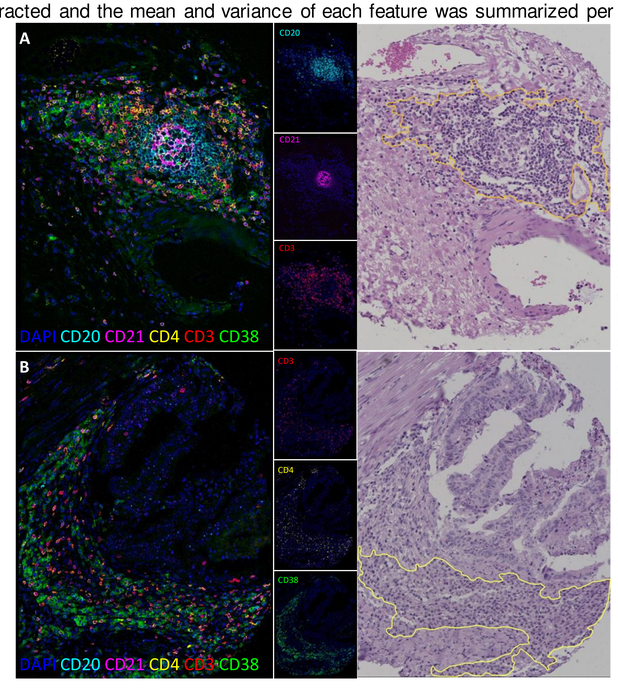

Figure 1. **(A)** *Left*: Representative mIF image with a mTLS. *Right*: Corresponding H&E slide with mTLS annotation. **(B)** *Left:* Representative mIF image with a lymphocyte aggregate. *Right:* Corresponding H&E slide with lymphocyte aggregate annotation.

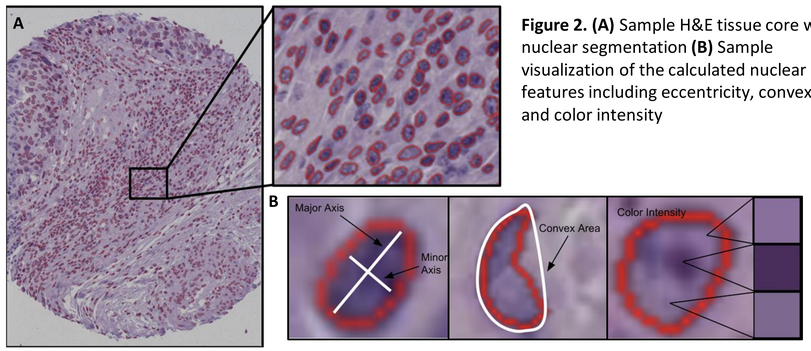

Figure 2. (A) Sample H&E tissue core with nuclear segmentation **(B)** Sample visualization of the calculated nuclear features including eccentricity, convexity, and color intensity

- Based on these features, a univariate analysis comparing mTLS and LA was performed using a Wilcoxon rank sum test, and a mTLS classifier was trained using a multivariate logistic regression model. The classifier performance was assessed using 5 repeats of 5-fold cross validation and average accuracy and area under the ROC curve (AUC) were calculated. Overall survival (OS) was compared between patients with at least one predicted mTLS (n=15), predicted LA (n=11) or none (n=9) using a log rank test and cox proportional hazards model.

RESULTS

- From the 140 tissue cores, we identified tissue cores with either mTLS (n=18), LA (n=34) or none (n=92). No core presented both TLS and LA.
- Cells in mTLS areas demonstrated higher mean nuclear eccentricity ($p<0.0001$) and solidity ($p=0.01$) along with lower variance in these features ($p<0.0001$ and $p=0.001$, respectively) compared to cells in LA areas.

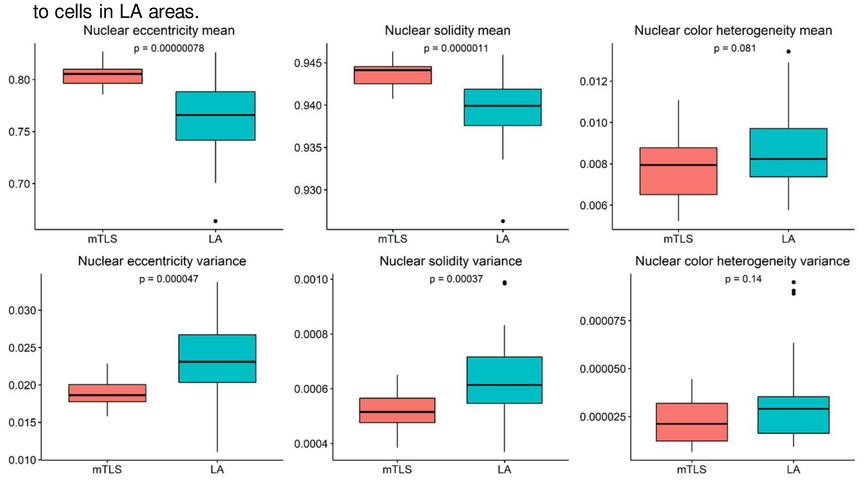

Figure 3. Differential expression of sample nuclear features including nuclear eccentricity, nuclear solidity, and nuclear color heterogeneity.

- A multivariate classifier trained on nuclear features exhibited 89% accuracy ($p<0.0001$) and 94% AUC ($p<0.0001$) in differentiating between mTLS and LA.
- Median OS was significantly higher in patients with at least one predicted mTLS (n=15) vs. patients with at least one predicted LA (n=11) detected on H&E (NR vs. 19 months, HR=0.14, 95% CI 0.036-0.54; p=0.0018).

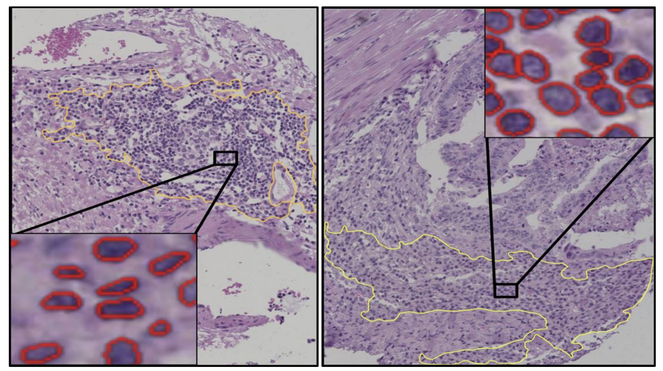

Figure 4. Representative images of nuclear morphology of cells in mTLS (*left*) and LA (*right*)

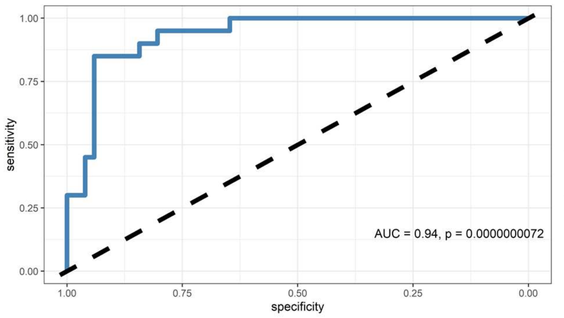

Figure 5. ROC curve of the multivariate mTLS vs. LA classifier trained on nuclear features

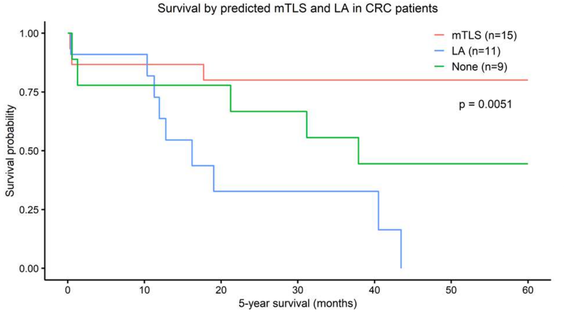

Figure 6. Kaplan-Meier curve demonstrating difference in clinical outcome (OS) of CRC patients with predicted mTLS compared to those with predicted LA or neither using log rank test

CONCLUSIONS

- Nuclear based morphological features can be used to accurately detect the presence of mTLS and LA from H&E slides, without the need for mIF or IHC stainings.
- Given the predictive value of mTLS presence, this work demonstrates the potential for mTLS detection in H&E slides to be used as a potential biomarker for patient selection for immunotherapy treatments.

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