Automatic quantification of TSR as a prognostic marker for pancreatic cancer.

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Abstract

The current diagnostic and outcome prediction methods for pancreatic cancer lack prognostic power. As such, identifying novel biomarkers using machine learning has become of increasing interest. In this study, we introduce a novel method for estimating the tumor-stroma ratio (TSR) in whole slide images (WSIs) of pancreatic tissue and assess its potential as a prognostic biomarker. A multi-step strategy for estimating TSR is proposed, including epithelium segmentation based on an immunohistochemical reference standard, a coarse pancreatic cancer segmentation, and a post-processing pipeline for TSR quantification. The resultant segmentation models are validated on external test sets using the Dice coefficient, and additionally, the TSR's potential as a prognostic factor is assessed using survival analysis, resulting in a C-index of 0.61.

Keywords: computational pathology, pancreatic cancer, survival, tumor-stroma ratio.

1. Introduction

Despite histopathology analysis of biopsies being the gold standard for Pancreatic ductal adenocarcinoma (PDAC) diagnosis, current histopathological biomarkers have limited predictive ability in determining prognosis. Furthermore, the TNM staging system itself, based on grading the depth of the invasion, number of metastatic nodes, and the status of other distant metastases, suffers from the same issues since patients with the same TNM stage present different prognoses (Edge and Compton, 2010; Song et al., 2018). Therefore, there is a need to develop reliable biomarkers that can better correlate tumor characteristics with survival to allow for better patient management.

The tumor-stroma ratio (TSR) represents the relative amount of tumor cells and intratumoral stroma and is a widely studied prognostic factor. In various solid tumors (Roeke et al., 2017; Zhang et al., 2015; Geessink et al., 2019; Scheer et al., 2017), TSR was identified as an independent prognostic factor. However, in pancreatic cancer, the role of TSR in predicting survival has been inconsistent with Leppänen et al. (2019) stating that it is not a reliable biomarker, and Li et al. (2020) assessing instead its predictive power. A

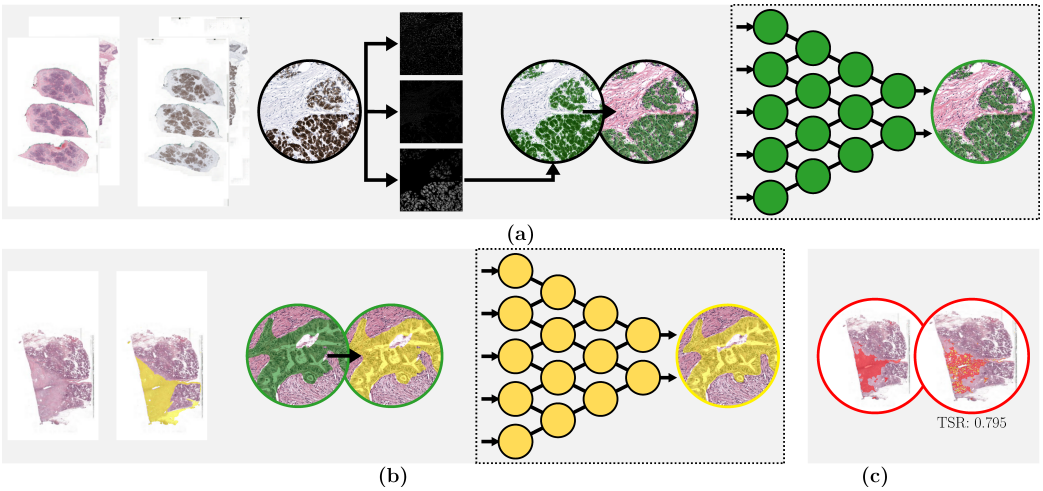

Figure 1: Flowchart highlighting different pipeline steps: (a) Epithelium segmentation and (b) tumor epithelium segmentation. Through the process of staining-destaining of paired H&E and IHC slides, epithelium annotations are obtained, which are then used to train an epithelium segmentation network. This network annotates the rest of the slides. Subsequently, a tumor epithelium segmentation network is trained on the segmented epithelium combined with annotated tumor area. Based on tumor epithelium segmentation, the tumor bulk is determined using the convex hull (c), on which TSR is calculated.

key point for this might be partly due to the variability in estimating the TSR by human observers. Geessink et al. (2019) have previously shown that using machine learning methods to estimate TSR in colorectal cancer had the potential to achieve more reproducible TSR estimates with prognostic power. This article proposes to assess the prognostic power of TSR in pancreatic cancer using a multi-step CNN-based pipeline for automatic tumor segmentation and TSR estimation (Figure 1).

2. Methods

Our study aimed to complete two main tasks: 1) pancreatic tumor segmentation and separation into epithelial and stromal components, and 2) TSR quantification and relating it to patient survival.

To complete these tasks, we used multiple datasets, including two internal datasets from Radboudumc, a publicly available dataset from TCGA-PAAD, and a private multicentric dataset gathered in collaboration with 24 other centers. A complete overview of the datasets containing origin, number of slides and number of cases is reported in Table 1.

For Task 1, we developed a two-step method for automatic tumor segmentation in WSI of the pancreas. In the first step, we trained a U-Net model (Iakubovskii, 2019) with a depth of five for epithelium segmentation on H&E slides. The model was trained using a reference obtained through a stain-restain procedure. The slide was first stained with H&E and digitized, then restained with cytokeratin (ck8/18) and digitized again at a resolotuon of 0.25μm. A color deconvolution technique was used to segment the epithelium, which was

then mapped to the H&E slide using a registration algorithm. The U-Net was then trained on H&E with the cytokeratin reference standard (Figure 1a) at a resolution of 1.0μm.

To subsequently obtain the separation between cancerous epithelium and other tissue we combined the epithelium segmentation results with coarsely drawn tumor annotations. This resulted in detailed annotations of the tumor epithelium. We trained another five-depth U-Net model to segment the tumor epithelium and evaluated its performance using Dice coefficient on both the internal/external datasets and the TCGA dataset. Last, by applying the alphahull algorithm to the resultant tumor epithelium segmentation we obtain both the detailed segmentation of the tumor cells and the full tumor area.

Dataset	Source	Patients [Slides]	Epi. Segm.	Tumor Epi. Segm.	Tumor Segm.
A	Radboudumc	16 [16]	0.749 (0.3)		
B	Multicentric	162 [162]		0.642 (0.254)	0.7 (0.27)
C	Radboudumc	29 [29]		0.751 (0.15)	0.76 (0.109)
D	TCGA-PAAD	161 [187]	0.717 (0.33)	0.726 (0.25)	0.863 (0.174)

Table 1: Dataset description with Dice coefficients for the various tasks.

For Task 2, we applied a multi-class tissue segmentation network (Bokhorsta et al., 2021), pre-trained on a colorectal tissue dataset, to the tumor area, which, among others, specifically segments stroma. By combining the full tumor area with the resultant stromal segmentation, we could calculate the TSR for each slide, and we quantified it by calculating the ratio of stromal components with respect to the whole tumor area. We performed then survival analysis by training a Logistic Regression model incorporating TSR and clinical variables in a five-fold cross-validation fashion on the TCGA dataset. We validated the model using the C-index, predicting patient survival at six month post-surgery. The clinical variables we considered for combining with the TSR were Age, Gender, Origin of the tumor, Primary diagnosis and Prior malignancy.

3. Results and Discussion

Table 1 shows a summary of the results for Task 1 for each of the datasets. The results of the segmentation show the robustness of both the epithelium segmentation and the tumor epithelium segmentation. The average median Dice for the epithelium segmentation is 0.733 (0.3 IQR), while the tumor epithelium segmentation has an average median Dice of 0.71 (0.22 IQR). The results for the tumor segmentation also show good performance, with an average median Dice of 0.77 (0.18 IQR). Survival analysis shows that baseline (clinical variables alone) have an AUC of 0.60 ± 0.12, while TSR in combination with clinical features improves performances in estimating 6-month survival, with an AUC of 0.61 ± 0.12.

In our study, we developed a fully automated method for TSR quantification through a multi-step CNN. Results of the various tasks show reliable performances and survival analysis shows evidence of prognostic power for the TSR. Li et al. (2020) proposed a similar method, but in comparison to them, we were able to fully automate the process, reducing the need for human supervision. Despite the promising results in the cross-validation, in future research, we will fully validate the prognostic relevance of the TSR in expanded cohorts of the patients.

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