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Investigation of the desmoplastic peritumoral microenvironment in cervical cancer

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Background

Desmoplastic alterations in the tumor microenvironment of cervical carcinoma are characterized by an increased deposition of collagen fibers by activated cancer-associated fibroblasts (CAFs) and are associated with malignant progression. The extent of desmoplasia is conventionally determined by pathologists, evaluating Hematoxylin and Eosin (HE) stained tumor tissue samples, and categorizing it on a scale from 0 (absent) to 3 (strong presence of desmoplasia)(**Fig.1**). Multiple studies investigating the pathomechanism of desmoplasia have been conducted on other solid cancers, highlighting the role of the Angiotensin-II-type 1 Receptor (AT1R) in CAF regulation.

The aim of this retrospective study is to establish a better understanding of desmoplastic remodeling in cervical cancer and further improve classification by usage of AI based quantification, possibly improving the identification of high and low risk groups.

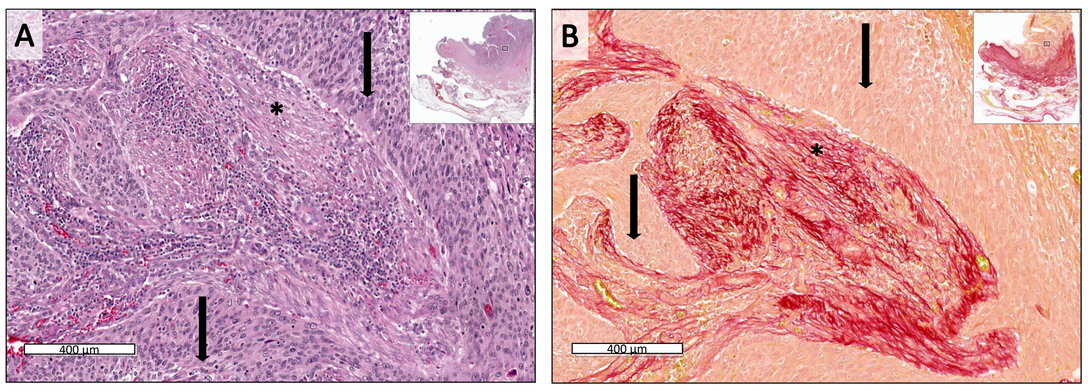

Fig. 1 Serial sections of the same cervical carcinoma with desmoplasia grade 3. A. The section was stained with HE. **B.** The section was stained with Picro-Sirius Red (PSR). PSR stain highlights collagen fibers red, particularly type I and III. Tumor cell clusters are indicated by an arrow (↓), desmoplastic areas are marked with an asterisk (*).

Methods

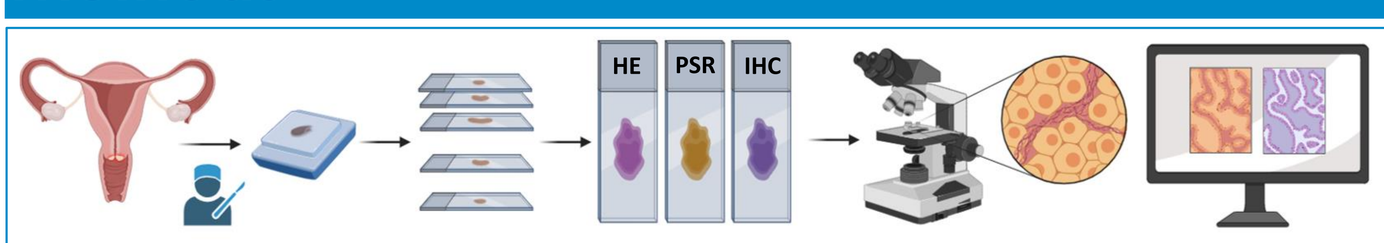

Fig. 2 Illustration of the sample preparation process. After surgical tumor resection, tissue samples were routinely paraffin-embedded and later sectioned into 5µm thick serial slices. To quantify the extent of desmoplasia precisely, we performed Picro-Sirius red, HE staining and Immunohistochemistry (IHC) of tissue specimen of each patient. The tissue slides were digitalized with an Aperio Verso 8 microscope and subsequently subjected to image analysis.

Tumor tissue of 100 patients with stage I or stage II cervical carcinoma was collected between 2015 and 2022 at our department (**Table 1**). After the sample preparation process (**Fig.2**), the digitalized tissue slides and deep learning methods were used to develop an algorithm in QuPath (0.4.4), that quantifies desmoplasia based on collagen staining (PSR based)(**Fig.3**) in comparison to conventional classification by pathologists (HE based). Additionally, we performed double immunohistochemistry using an antibody against the AT1R along with staining for the CAF subtype marker a-smooth muscle actin, to investigate the involvement of the AT1R in the regulation of CAFs in cervical carcinoma.

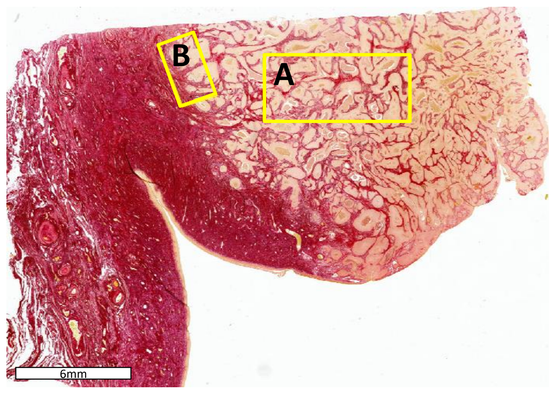

Fig. 3 Overview Image of a PSR-stained cervical carcinoma A. The intratumoral region of interest that is analyzed by our algorithm is individually determined in collaboration with pathologists. **B.** If possible, a second region of interest is analyzed in the tumor invasion front.

Histology	n patients without desmoplasia (grade 0)	n patients with desmoplasia (grade 1-3)
Squamous cell carcinoma	27	49
Adenocarcinoma	6	18

Table 1 Tumor characteristics of included patients (n=100).

Results

To simplify the analysis, our algorithm has been exclusively trained on squamous cell carcinoma up to this point. The algorithm is used to collect three distinct metrics: the **tumor-stroma ratio**, **collagen content** and **collagen density** (**Fig. 4**). All measurements are expressed in percentage. Preliminary data from 40 analyzed patient samples are visualized in **Figure 5**. Protocols for AT1R and CAF marker staining were established using validated controls. The staining and digitalization process is in progress and the images will be analyzed afterward.

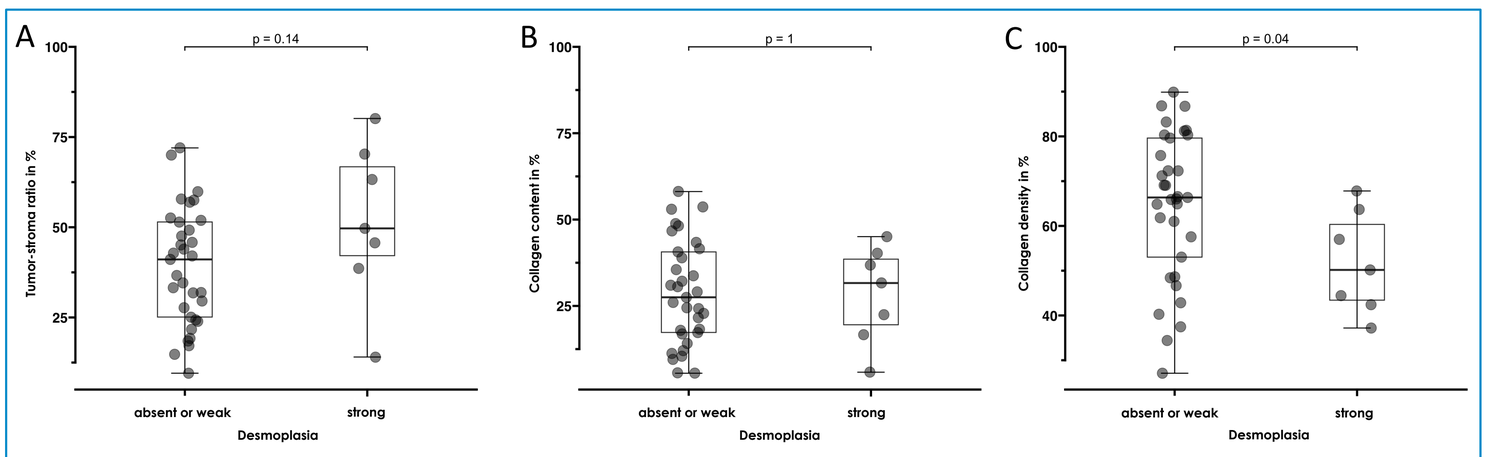

Fig. 5 Boxplots illustrating preliminary data from 40 analyzed patients with squamous cell carcinoma. The patients are divided into groups with absent or weak demoplasia (n=33) and strong desmoplasia (n=7). Each data point represents the measurement of the intratumoral region analyzed by our algorithm for one patient. **A. Tumor-stroma ratio:** A trend toward higher collagen content in individuals with higher grades of desmoplasia is depicted. This association is not yet significant. **B. Collagen content:** No significant difference between the two groups is shown. **C. Collagen density** The analysis shows higher collagen density in patients with higher grades of desmoplasia compared to patients with absent or weak desmoplasia. The observed association is statistically significant for this measurement ($p<0.05$).

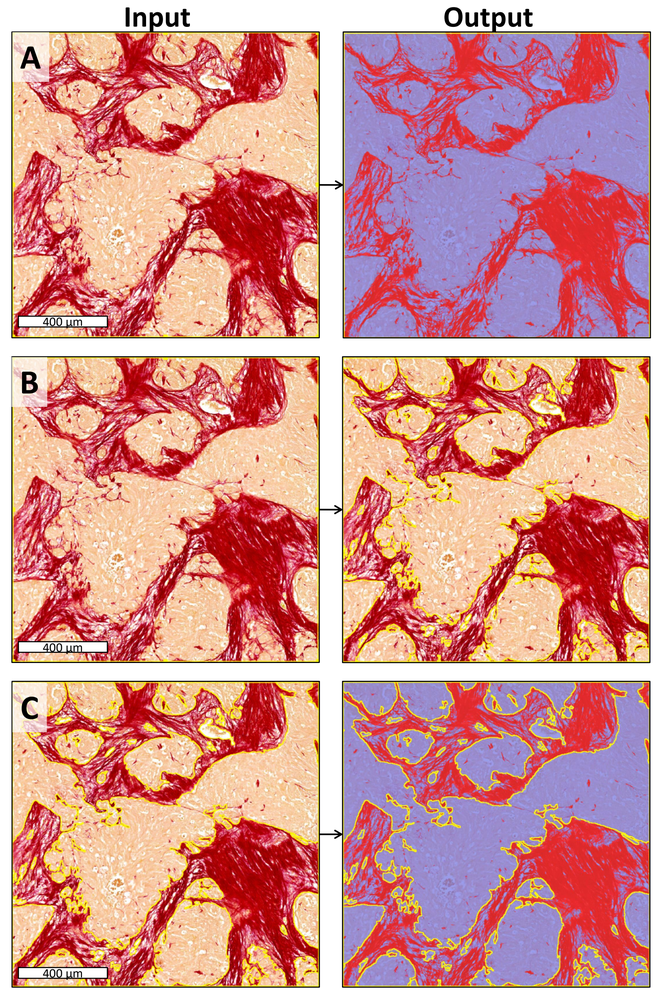

Fig. 4 Analyzed PSR-stained section of a cervical carcinoma with moderate desmoplasia (grade 2). On the left side, the image excerpts before analysis with our algorithm are displayed, while on the right side, the respective output of each analysis is shown.
A. Measurement of collagen content: A colour threshold is applied to measure the area of collagen fibers (red) in relation to the total area of the analyzed section.
B. Measurement of tumor-stroma ratio: A machine learning algorithm is employed to identify the stromal area (highlighted in yellow underline). The stromal area is measured in relation to the total area of the analyzed section.
C. Measurement of collagen density: After applying the machine learning algorithm to identify the stromal area (highlighted in yellow underline), a colour threshold is then applied to the stromal area, measuring the area of collagen fibers (red) in relation to the stromal area.

Discussion

The findings elucidate the potential enhancement of demoplasia assessment and quantification through our algorithm, particularly by gauging collagen density in PSR-stained slides. When assessing the results, it is important to consider the small sample size, especially with a limited number of patients with strong presence of desmoplasia.

Outlook

- Following the evaluation of squamous cell carcinoma, we plan to expand the patient samples to include adenocarcinomas.
- We plan on correlating the results with clinical outcomes, such as survival and lymph node infiltration.

AI based quantification of desmoplasia could improve manual assessment by pathologists. Objective estimation gains significance, especially if our research shows a correlation between AT1R expression of CAFs and the extent of desmoplasia, providing a basis for targeted therapeutic intervention at the AT1 receptor as a new therapeutic approach.

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