AUTOMATED QUANTITATIVE ANALYSIS OF EPITHELIAL AND STROMA AREA IN COLORECTAL CANCER: Putative Application for the Prognosis of Cancer Progression

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BACKGROUND

In tumour progression, the microenvironment is altered and the tissue architecture and homeostasis is lost. A decrease in the ratio of epithelial vs. stromal area is considered an indicator for epithelial-mesenchymal transition which favours metastasis formation (1) by the activation of cell proliferation pathways and a decrease in cell adhesion protein expression. Recently, the epithelium/stroma ratio was proposed to be a prognostic factor in breast (2) and colorectal cancer (CRC) (3). However, in these studies visual estimation was done by two or more experts, which is a tedious, subjective and error-prone method. Based on cutting edge imaging technologies, the automatic detection of large tissue areas and subsequent automatic image processing of immunostained tissue allow an accurate, fast and reproducible analysis of tissues. In future, this technology may contribute to better classification of disease and prognosis prediction.

AIM

We aimed to develop image processing algorithms that allow an automatic detection and quantification of the epithelial and stromal area in colorectal cancer tissue and to compare the epithelium/stroma ratio between patients with and without liver metastasis.

RESULTS

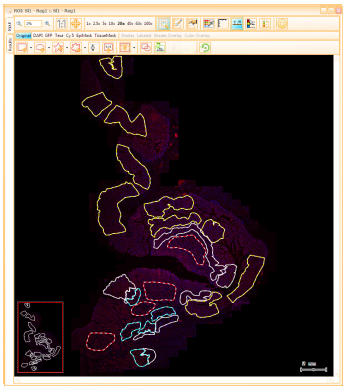


Figure 1 – Regions of interest defined in the virtual slide after scanning

We performed immunofluorescence staining of Keratin 8 (K8) for epithelial cell identification on paraffin-embedded sections from 10 patients with colorectal cancer grade 2. Six patients had already developed liver metastasis.
After the scanning of the stained sections, the regions of interest were manually defined in all virtual slides using annotation tools in TissueQuest. The software allows the results of multiple regions to be merged into custom groups, defined as the following: tumour centre (red), invasive front (cyan) and adjacent mucosa (yellow) (**Fig. 1**).

Figure 2 – Image processing workflow (a) and subsequent generation of the epithelial and stromal masks (b) from the immunofluorescence staining (c)

Fig. 2a

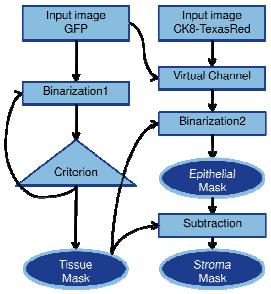


Fig. 2b

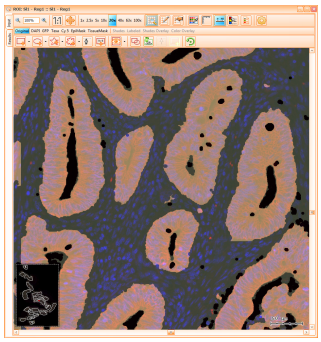
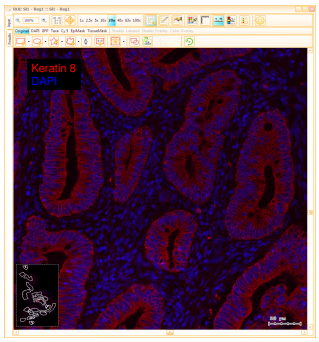
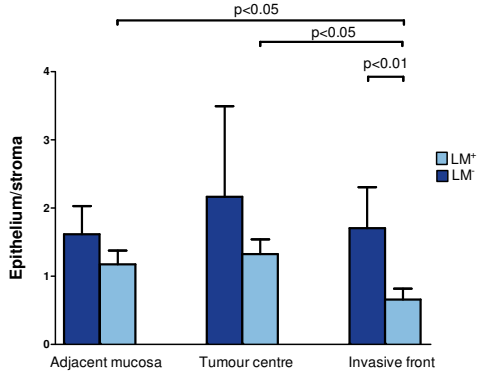


Fig. 2c



The image processing used a multi-channel virtual slide as input that contained information of total tissue autofluorescence from GFP and epithelial tissue from K8-TexasRed channels (**Fig. 2c**). A restricted binarization method applied on the preprocessed GFP images was used to discriminate the tissue area from the lumen. Then the epithelial area was detected on a 'virtual channel' formed by combining intensity and gradient information from all available channels, as well as from the tissue mask realized in the previous step. Finally, the stroma representing the remaining part of the image was measured by subtracting the epithelial area from the tissue area (**Fig. 2a**). **Fig. 2b** shows the epithelial (orange) and stromal (grey) mask.

Figure 3 – Epithelium/stroma ratio in different sub-regions in colorectal cancer patients with and without liver metastasis



At the invasive front, CRC patients with liver metastasis (LM^+) showed a strong decrease, up to 61%, in the epithelium/stroma ratio compared with patients without liver metastasis (LM^-) ($p<0.01$). A decrease in the ratio was also found when comparing the invasive front with the tumour centre and the adjacent mucosa within LM^+ patients ($p<0.05$).
Moreover, at the tumour centre, a tendency towards a lower epithelium/stroma ratio was found in LM^+ compared with LM^- patients. Although the ratio at the tumour centre and the adjacent mucosa of LM^+ patients is similar, previous studies reported differences in the cellular and soluble components of the stroma. The increase in the epithelial area at the tumour centre of LM^- patients when compared with the adjacent mucosa (n.s) is due to the uncontrolled growth of epithelial cells and thus a reduction of the stromal area. The high variability observed at the tumour centre of LM^- patients may be a reflection of the heterogeneity of this G2 group.

METHODS

Immunofluorescence staining: Anti-Keratin-8 (clone EP1628Y Thermo Fisher Scientific) was used as primary antibody followed by DyLight 549 (Vector) secondary antibody. Nuclei were stained with DAPI.
Tissue analysis: Large areas of tumour tissue (150-300 mm^2) were scanned with a 20x objective using the automated microscopy system TissueFAXS™ (TissueGnostics GmbH). For every section we defined sub-regions (0.3-36.2 mm^2) for invasive front, tumour centre and adjacent mucosa. The images were analysed by TissueQuest (TissueGnostics) analysis software using classical image processing algorithms optimized for large images.
Statistical analysis: Results are presented as mean and SD. The Wilcoxon-Mann-Whitney test was applied for unpaired comparison, the Wilcoxon signed rank test for paired comparison. A p-value<0.05 was considered statistically significant.

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CONCLUSIONS AND OUTLOOK

1) **The strong reduction of the epithelium/stroma ratio at the invasive front of colon cancer tissue of patients with liver metastasis confirms the benefit to use this parameter as an additional information in CRC diagnosis.**
2) **Our results are in agreement with previous studies that used visual methods.**
3) **The implemented software can be used for detailed morphometric analysis of CRC tissue, as well as for the quantitative evaluation of markers expressed in the epithelial or stromal area for a better characterization of epithelium-stroma interaction in tumour progression.**

REFERENCES

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