Identification of high risk patients in stage II pT3N0 microsatellite-stable colorectal cancer

E. Budinská [1] T. Catela-Ivković [2] M. Hrivňáková [1] O. Slabý [2,3] B. Bencsiková [4] V. Popovici [1]

[1]RECETOX, Fac. of Science, Masaryk University, Brno, CZ [2]CEITEC, Masaryk University, Brno, CZ [3]Fac. of Medicine, Masaryk University, Brno, CZ [4]Masaryk Memorial Cancer Institute, Brno, CZ

EACR 2023 12 - 15 June 2023 Torino, Italy

Goal: To identify early colon cancer patients with high risk of relapse.

Motivation

The risk of recurrence of patients with stage II colorectal cancer (CRC) without adjuvant chemotherapy is, in general, 15-20%. It is estimated that about 2% of the general population of stage II patients benefit from adjuvant chemotherapy. In the absence of other risk factors, patients with stage II pT3N0 CRC are considered low risk and not given adjuvant chemotherapy. However, about 10% of these patients relapse within five years. Here, we identify the main risk of relapse markers for pT3N0 CRC patients which were not selected for adjuvant chemotherapy.

Study design

Inclusion criteria:

- stage II, pT3, good prognosis by standard clinico-pathologic parameters
- no lymphovascular invasion
- no bowel perforation (to the extent of available data)
- microsatellite-stable (MSS)
- no adjuvant therapy

Data:

- two data collections: MUNI ($n = 39$) and E-MTAB-863[1] ($n = 150$)
- **early relapse group** was defined as relapse occuring within 5 years, while **no relapse group** was defined as no relapse for at least 6 years
- initial staging was re-confirmed at the time of sample retrieval from the biobank (MUNI dataset)
- MUNI: no relapse: $n = 19$, early relapse: $n = 20$; E-MTAB-863: no relapse: $n = 94$, early relapse: $n = 56$
- RNA was extracted from FFPE sections and profiled using Thermo Fisher Clariom™ D Human assay

Methods

- differential gene expression using LIMMA
- gene set enrichment analysis with MSigDB v7 signatures
- predictor: logistic regresson with Elastic Net regularization
- repeated ($10\times$) stratified 5-fold cross validation was used for performance estimation
- Agresti-Coull 95% confidence intervals for performance parameters

Results: BRAF-mutant-like tumors

Since the *BRAF* mutation status was unknown, we tested whether the *BRAF*-mutant-like signature [2] may provide any insights.

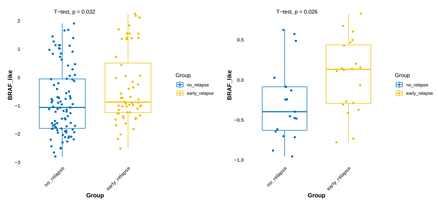

Figure 1. BRAF-mutant-like scores in the two datasets.

Results: genes and pathways

Hallmark pathways

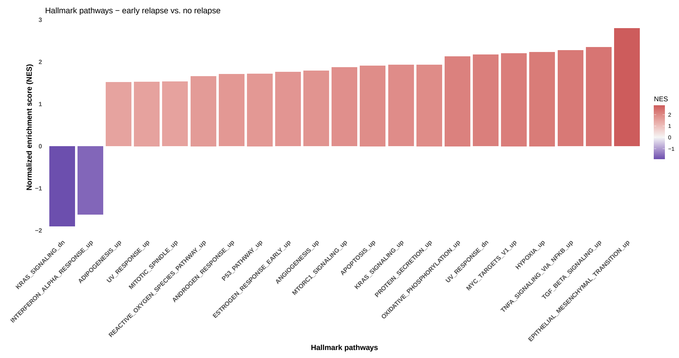

Figure 2. Hallmark pathways scores. All scores are shown as normalized scores and only statistically significant ($p \leq 0.01$) pathways/signatures are listed.

- EMT: main genes: ECM2, IL6, CCN{1,2}, TGFBI, POSTN, VIM, SERPINE{1,2}, COL{5A1, 5A2,3A1, 11A1, 1A1, 4A1, 4A2}, CDH11, CXCL{1,8}, ...
- TGFβ signaling genes: THBS1, ID2, BMP{2,R1A,R2}, SKIL, SMURF{1,2}, SMAD{3,6}, TGFBR1, CDH1, ...
- KRAS signaling, genes UP: TFPI, PTPRR, CXCR4, MAP3K1, PLAUR, DUSP6, TSPAN13, ...; and genes DOWN: SLC25A23, GP2, UGT2B17, CPA2,...

Cancer-associated fibroblasts and other signatures

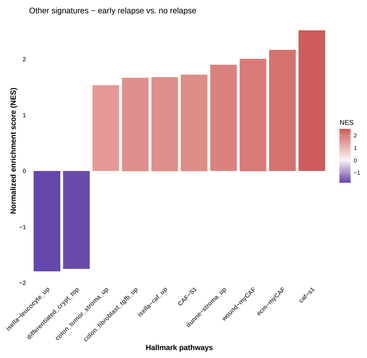

Figure 3. Stroma and CAF-related gene signatures. Only statistically significant ($p \leq 0.01$) signatures are shown.

- CAF-S1 [3] - associated with immunosupressive microenvironment and pro-metastatic functions: POSTN, CDH11, MMP19, CD55, CXCL{1,12},...
- Dunne stroma signature [4]: FABP4, IGF1, SFRP{1,2}, CXCL{1,5,8}, TGFB3,...

Results: gene expression-based predictor

- Elastic Net predictor with $\alpha = 0.4$ and $\lambda = 0.03$
- performance on MUNI dataset: **Accuracy** $= \mathbf{76.9}\%(95\%\mathbf{CI} : \mathbf{60.3} - \mathbf{88.3})$
- performance on E-MTAB-863 dataset: **Accuracy** $= \mathbf{71.33}\%(95\%\mathbf{CI} : \mathbf{63.3} - \mathbf{78.3})$
- **Precision** (identification of high risk patients): $\mathbf{80}\%(95\%\mathbf{CI} : \mathbf{55.7} - \mathbf{93.4})$ and $\mathbf{73.2}\%(95\%\mathbf{CI} : \mathbf{59.5} - \mathbf{83.8})$, respectively (threshold not optimized)
- top genes selected in the model: CEP112, MRAP2, MED24, OLFM1, CYP27B1, ARHGAP24, BEND7, USP50, TMEM132C, PDE5A, MRPL15, ST6GALNAC5, CD33, SYTL5, FGD3, LSS, CLEC4A, B9D1, TFPI, RNF169

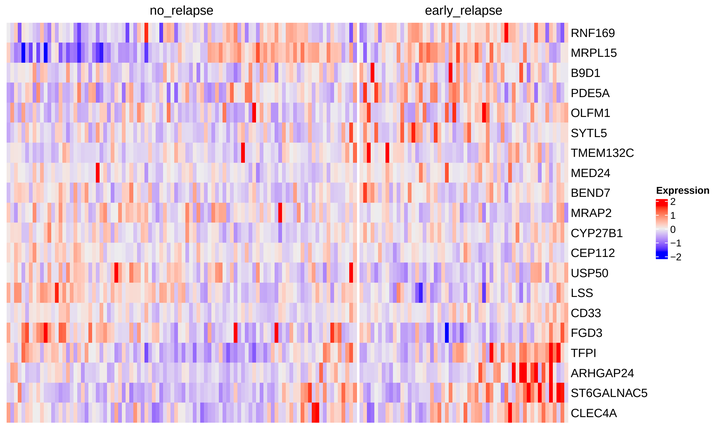

Figure 4. Heatmap based on top 20 genes from the Elastic net model.

References

1. Kennedy RD, *et al.* Development and independent validation of a prognostic assay for stage II colon cancer using formalin-fixed paraffin-embedded tissue. JCO 29(35), 2011.
2. Popovici V, *et al.* Identification of a poor-prognosis BRAF-mutant–like population of patients with colon cancer. JCO 30(12), 2012.
3. Pelk K, *et al.* Spatially organized multicellular immune hubs in human colorectal cancer. Cell184(18), 2021.
4. Dunne PD, *et al.* Cancer-cell intrinsic gene expression signatures overcome intratumoural heterogeneity bias in colorectal cancer patient classification. Nat Commun 8:15657, 2017.

Acknowledgements

This project was supported and funded by Agentura pro Zdravotnický Výzkum České Republiky (AZV) – Grant Agency of the Czech Ministry of Health – under grant no. NV19-03-00298. Part of the work was carried out with the support of core facilities of RECETOX Research Infrastructure, project number LM2023069, funded by the Ministry of Education, Youth and Sports of the Czech Republic under the activity „Projects of major infrastructures for research, development and innovations".