Quantitative measurement of functional activity of the TGFβ and MAPK-AP1 pathways in colon cancer provides information on their role in cancer development and metastasis

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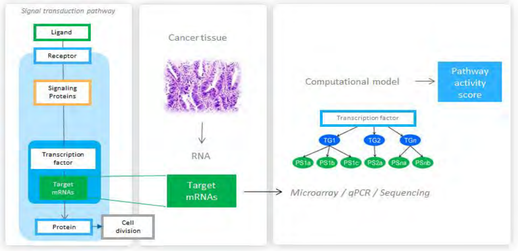
Background. Use of targeted drugs for blocking tumor driving signaling pathway(s) leads to improved outcome for cancer patients. However, a critical requirement is accurate measurement of signaling pathway activity in a cancer tissue sample. Novel RNA-based assays enable simultaneous and quantitative measurement of signaling pathway activity in tissue samples [1]. Using these assays, we previously reported that Wnt [1] and PI3K pathways [2] become active in colon carcinomas.

Methods. Affymetrix U133 Plus 2.0 expression microarray datasets from the Gene Expression Omnibus were used to investigate activity of MAPK-AP1, and TGFβ pathways in colon cancer in general, and in the Consensus Molecular Subtypes (CMS) [3].

Assays for quantitative measurement of signal transduction pathway activity

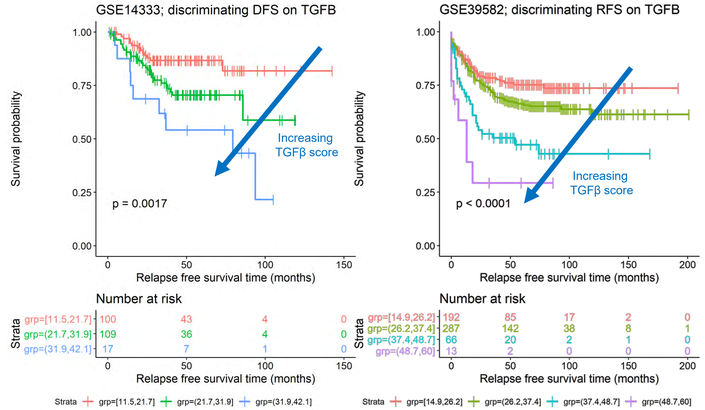
RNA-based tests have been developed for androgen (AR) and estrogen receptor (ER), Hedgehog (HH), Wnt, TGFβ, Notch, NFκB, PI3K, and MAPK-AP1 pathways. They can be performed simultaneously on a single tissue or cell culture sample using Affymetrix HG-U133 Plus 2.0 microarray, qPCR or RNA-sequencing and provide quantitative pathway activity scores expressed on a 0-100 scale [1-5].



Increased TGFβ pathway activity in primary tumor is related to reduced relapse/disease free survival time

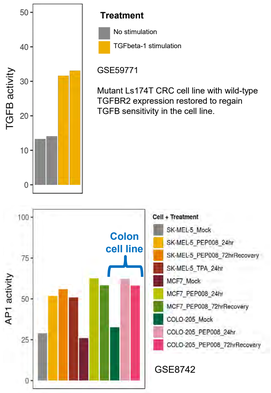
In two independent datasets higher TGFβ pathway activity was associated with reduced relapse/disease free survival.

MAPK-AP1 pathway activity was not significantly associated with outcome.



TGFβ pathway activity assay

The TGFβ assay was described before [4]. Validation for use on colon tissue: in a TGFβ-sensitive colon carcinoma cell line, stimulation with TGFβ increased pathway activity (GSE59771).



MAPK-AP1 pathway activity assay

The MAPK-AP1 assay was developed as described [1]. Validation for use on colon tissue: in the colorectal adenocarcinoma cell line COLO-205, the phorbolester PEP008 induced an increase in MAPK-AP1 pathway activity.

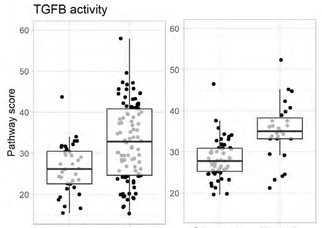
TGFβ pathway activity is increased in colon cancer metastases

TGFβ activity in metastases showed large variations between individual patients.

On average, TGFβ activity is higher in metastases compared to primary tumor.

Two-sided t test:

p=2.7e-6 for GSE41568
p=5.0e-5 for GSE28702

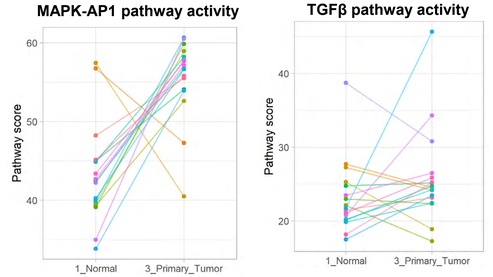


MAPK-AP1 and TGFβ pathway activity in matched normal and primary tumor colon tissue

MAPK-AP1 pathway activity increases with two exceptions. This is in line with frequent KRAS mutations that activate this pathway [6].

On average, TGFβ pathway activity is slightly elevated, but not significant (t-test p=0.18).

Note: samples were not microdissected and therefore contain a mixture of cell types. This may impact the findings.

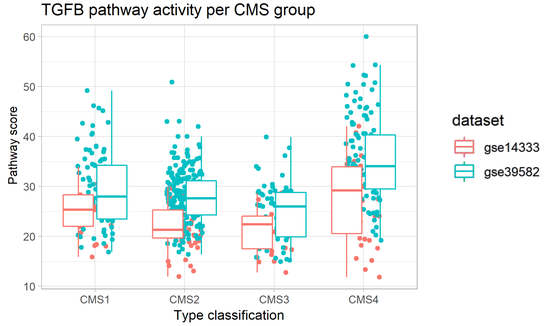


Lines connect matched samples, per patient the same color
Both figures: GSE110224.

Association between TGFβ pathway activity and CMS classification

Two independent datasets: Higher TGFβ pathway activity was associated with CMS4. This complements the reported association of CMS4 with TGFβ pathway-related genes [7].

Differences between datasets in absolute TGFβ pathway activity scores are probably caused by sample preparation protocol differences (different QC parameters observed).



Discussion / Conclusion

- **High TGFβ pathway activity in primary tumor is associated with reduced relapse/disease free survival time; and TGFβ pathway activity increased further in metastases. These results suggest the TGFβ pathway as a potential therapeutic target, in those patients with high pathway activity.**
- **MAPK-AP1 pathway activity is increased in colon cancer, but not related to clinical outcome.**
- **Future clinical studies: confirmation and exploration of the TGFβ pathway as a therapeutic target to prevent metastasis on a personalized basis.**
- **TGFβ and MAPK-AP1 pathway activity assays have been developed and validated for use on colon tissue.**

References

[1] Verhaegh W, et al. Cancer Res. 2014;74(11):2936–45.
[2] Verhaegh W, Van de Stolpe A. Oncotarget. 2014;5:5196–7.
[3] van Ooijen H, et al. Am J Pathol. 2018;188:1956–72.
[4] Stolpe A van de, et al. Sci Rep. 2019;9:1603.
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AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: October 26, 2019 - October 30, 2019