Exploring candidate signal transduction pathways for targeted therapy in esophageal cancer

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Aim

To reveal candidate signaling pathways for targeted therapy in esophageal cancer (EC), and esophageal adenocarcinoma (EAC) in particular, we investigated key signal transduction pathways in material available in clinical routine;

(i). before and after neoadjuvant chemoradiation (nCRT) according to CROSS

(ii). for primary tumor and recurrent disease

In addition, to explore the possibility to use patient-derived-xenografts (PDX) and (patient-derived) cell lines as a model to identify novel therapies

(iii). PDX of matched patients material were analyzed.

Methods

Digitally annotated tumor areas ≥2 mm2 were transferred to a consecutive hematoxylin-stained slide and scraped for RNA extraction. Subsequently, a panel of qPCRs was performed, to infer signal transduction activity of the AR-, ER-, PI3K- (inverse of FOXO), HH-,TGF-ß- and Wnt pathway.

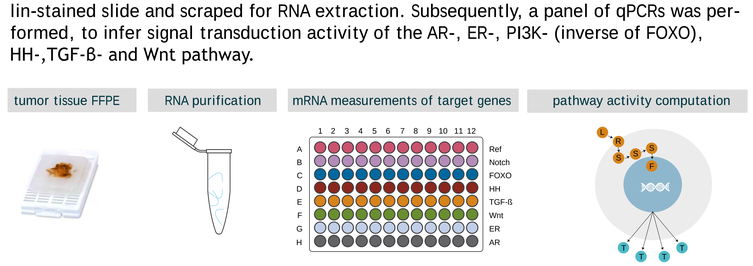

Fig. 1 Methods of pathway activity measurements in FFPE samples.

Increased PI3K activity in poor responders

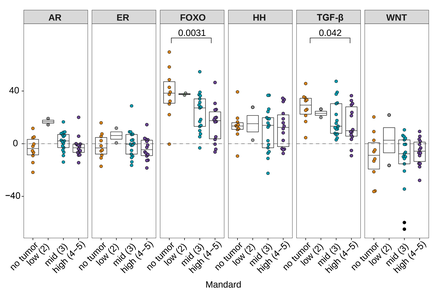

Fig. 2 The difference in pathway activity between pre-treatment tumor biopsies and corresponding post-nCRT tumor resection specimens (delta). A significant increase in PI3K- and decrease in TGF-B activity was observed in EAC poor- compared to good nCRT-responders. Note: the label no tumor is referring to no remaining tumor after nCRT, e.g. complete pathological response.

Poor nCRT-responders: association loss TGF-ß- and gain PI3K activity

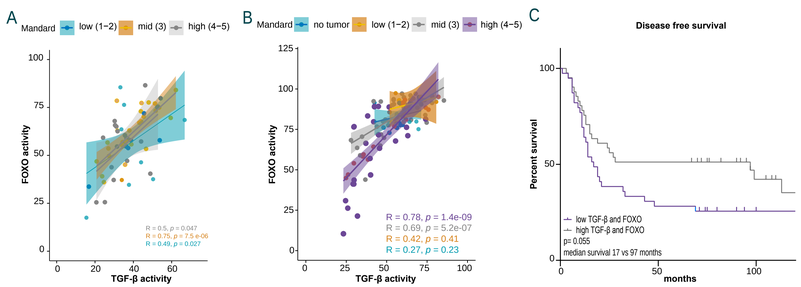

Fig. 3 Both for pre-treatment EAC biopsies (A) and post-nCRT EAC resection specimens (B) a correlation of FOXO (inverse of PI3K activity) and TGF-ß pathway activity is seen, suggesting a tumor suppressive role of TGF-ß. Low TGF-ß and high PI3K pathway activity is associated with worse response to nCRT in EAC. These findings are in line with the known tumor suppressive role of TGF-ß, and the tumor promoting role of the PI3K pathway. Patients with low TGF-ß and high PI3K pathway activity have worse survival compared to opposite activities in post-nCRT tumors (cut-off median: TGF-ß 56.3, FOXO 82.7) (C). A difference in median survival between 17 vs 97 months is seen, respectively.

Poor responder profile in recurrences

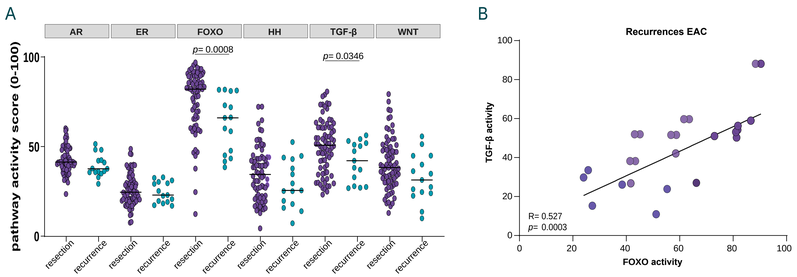

Fig. 4 A. Compared to nCRT treated resection specimen, recurrences are characterized by high PI3K- (low FOXO) and low TGF-ß pathway activity. This corresponds to the poor responder profile seen in post-nCRT treatment resection specimens. B. PI3K- and TGF-ß pathway activity also correlate in recurrences. High PI3K- is correlated to low TGF-ß activity.

Activity profiles conserved in PDX model

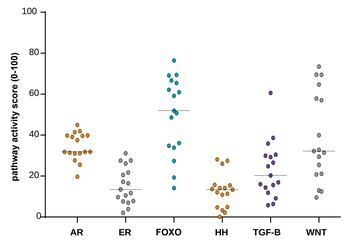

Fig. 5 Activity profiles of patient-derived xenograft models demonstrate similar activity profiles as FFPE patient material. Moreover, a range in PI3K signal transduction pathway activity is seen.

Patient derived cell lines as tool to study PI3K pathway targeted therapies in EC

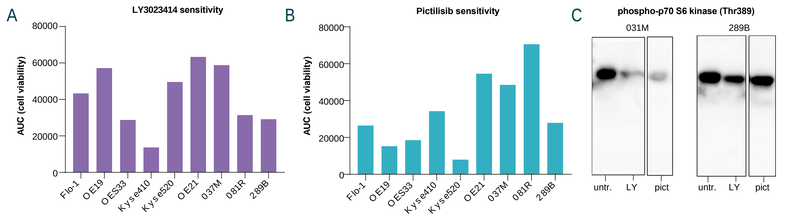

Fig. 6 EC cell lines were treated with PI3K-inhibition as add-on to standard nCRT. Using Cell Titer Blue as read-out for cell viability, the AUC was calculated; PI3K sensitive cell lines have a higher AUC. Varying PI3K inhibition sensitivities were observed for LY3023414 (A) and Pictilisib (B). This was also seen using western blot analysis in patient-derived cell lines treated with LY3023414 and pictilisib, immunoblotting for phospho-p70 S6 (Thr389) (Cell Signaling), a downstream PI3K-pathway target (C).

Conclusion

- **Loss of tumor-suppressive TGF-ß and concurrent high PI3K pathway activity are associated with poor response to nCRT in EAC.**
- **This poor-responder profile is preserved in recurrences of nCRT pre-treated patients and PDX models, providing a valuable tool to experimentally test targeting candidate signal pathways such as PI3K.**
- **It should be further investigated if PI3K-inhibition as add-on to standard nCRT treatment could improve outcomes in EC.**

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