Development of a Novel RNA-Based Fibroblast Growth Factor Receptor Response Signature (FGFR-PRS) for Use In Patients With Urothelial Cancer (UC)

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BACKGROUND

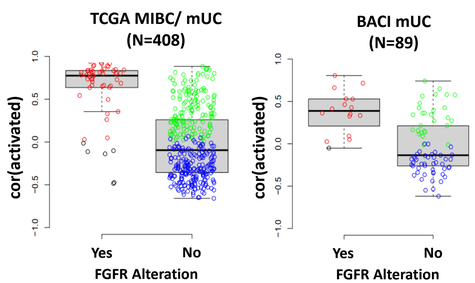
- FGFR-targeted (FGFRi) therapies are capturing clinical attention for treating UC following accelerated approval of erdafitinib in locally advanced/metastatic (m) post-chemotherapy UC patients with FGFR2/3 (i.e., DNA mutations and fusions) alterations (ALT)
- FGFRi being studied in ongoing clinical trials include erdafitinib (NCT05316155; NCT04172675; NCT03390504; NCT04083976), LOXO-435 (NCT05614739), and pemigatinib (NCT03914794)
- FGFR ALT status may not capture all patients who may show clinical benefit as responses are observed both in ALT (+) and (-) patients (Schuler et al., 2019; Choi et al., 2019)
- Improved test strategies are needed to identify patients most likely to respond to FGFRi
- Here we describe FGFR-PRS, an RNA-based gene expression classifier developed to capture the molecular phenotype of FGFR-active tumors independent ALT status and is intended to be used as a diagnostic test to identify a broader patient population likely to respond to FGFRi

METHODS

- Known oncogenic FGFR3 ALTs (S249C, R248C, FGFR3c-Y373C, FGFR3c-G370C, FGFR3:TACC3 and FGFR3:BAIAP2L1) were used as training labels for nearest centroid classifier development following the method of Dabney, 2005
- RNA sequencing (RNAseq) data from 2/3 of TCGA BLCA (TCGA) cohort, predominantly comprised of muscle-invasive bladder cancer (MIBC) and a few mUC, were used for training
- A resulting 80-gene classifier was applied to the remaining 1/3 of TCGA and a separate BACI mUC cohort (Rose et al., 2021) as test sets
- In vitro drug sensitivity was evaluated using data from the Genomics of Drug Sensitivity in Cancer database (GDSC; Yang et al., 2013; https://www.cancerrxgene.org/)
- Differential gene expression (DGE) gene network results were displayed using String (Szklarczyk et al., 2023; https://string-db.org/)

RESULTS

Figure 1: FGFR Alteration/FGFR-PRS Status in Bladder Cancer



Group Designation (boxplot dot color)	Status				Cohort (Bladder Cancer Stage)	
	FGFR Alteration		FGFR-PRS		TCGA (MIBC/mUC)	BACI (mUC)
	(+)	(-)	(+)	(-)		
A	✓		✓		49 (12%)	15 (17%)
B		✓	✓		149 (37%)	28 (31%)
C		✓		✓	204 (50%)	45 (51%)
D	✓			✓	6 (1%)	1 (1%)
				Total	408	89

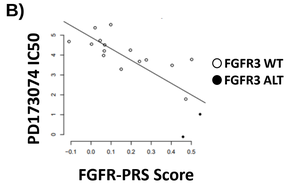
- Thirteen and 18% of the TCGA and BACI cohorts, respectively, were ALT (+), whereas 49% and 48% were FGFR-PRS (+) independent of ALT status (Figure 1)
- About half of the patients in either cohort were ALT (-)/ FGFR-PRS (-) and only 1% were ALT (+)/FGFR-PRS (-)

Figure 2: Association of Drug Sensitivity (IC50) and FGFR-PRS Score in Bladder Cancer Cell Lines Treated with FGFR Inhibitory Agents

A)

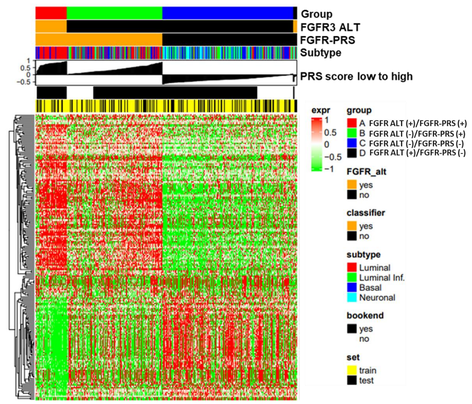
Drug Name	Putative Target (isoforms)	N	Spearman r	p value
PD173074	FGFR (1-3)	18	-0.75439	0.0005
FGFR_3831	FGFR (1-4)	19	-0.55439	0.0152
BIBF-1120	VEGF, PDGFR, FGFR (1-4)	19	-0.54737	0.0168
AZD4547	FGFR (1-3)	18	-0.46749	0.0522

B)



- FGFR 1-3 selective drugs had strong inverse correlations between FGFR-PRS score and IC50 across 18-19 GDSC bladder cancer cell lines (Yang et al., 2013) (Figure 2A)
- Representative correlation plot provided in Figure 2B

Figure 3: Heat Map of Top Genes Associated with FGFR3 Alterations and UC Subtypes



- Heat map constructed using the n=408 TCGA cohort and 209 genes associated with UC, FGFR3 alterations, and UC subtypes
- Primary grouping used the same A-D designations described in Figure 1
- Demonstrates that Group A and most of Group C have near consistent all up and all down gene expression profiles whereas B looks like A but noisier, suggesting that Group B shares an "FGFR3 active" phenotype

Figure 4: FGFR-PRS Gene Ontology Analysis of TCGA Cohort

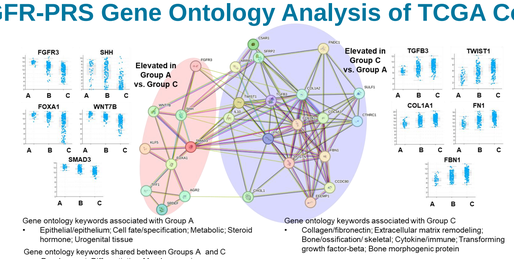
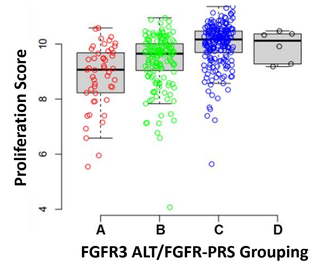


Figure 4: Cont.

- FGFR-PRS (+) samples (Group A and B) had enhanced co-expression of FGFR3, FOXA1, and SHH and genes with differentially enhanced expression in FGFR-PRS (+) pointed to epithelial/urogenital tissue-like ontologies
- FGFR-PRS (-) samples had elevated TGFB3, TWIST1, and COL1A1 expression that contributed to a bone-like phenotype associated with extracellular matrix remodeling and collagen/fibronectin proteins
- The A (FGFR3 high) and C (FGFR3 low) bookend groups, although defined by different enrichment gene sets, shared an enrichment of ontologies related to development, differentiation, and morphogenesis
- Group B (ALT (-)/ FGFR-PRS (-) tumors) shared characteristics of both Groups A and C but Group B gene medians for key genes tended to mirror more closely those in the group A network (e.g., FGFR3, SHH, etc.) than the group C network (e.g., TGFB3, TWIST1, etc.)

Figure 5: Relationship between Proliferation Score and FGFR3 ALT/FGFR-PRS in TCGA Cohort



- Primary grouping used the same A-D designations described in Figure 1
- Lower proliferation was observed in FGFR-PRS (+) groups, A and B, than in FGFR-PRS (-) groups, C and D, suggesting that FGFR activation may suppress proliferation

SUMMARY AND CONCLUSIONS

- FGFR-PRS is a gene expression classifier trained to identify tumors with an active FGFR3 expression phenotype irrespective of FGFR3 ALT status
- FGFR-PRS (+) captured most ALT (+) tumors and an additional 2X more with similar FGFR pathway activation
- FGFR-PRS (+) tumors were associated with gene enrichments for ontologies linked to FGFR3 signaling
- The correlation of FGFR-PRS score with in vitro FGFRi activity provided initial utility of the classifier, which is undergoing clinical evaluation in the ALAMANCE retrospective study of UC patients treated with FGFRi or other standard-of-care therapies
- Analytical validation is ongoing to support FGFR-PRS for use as a clinical trial assay and eventual diagnostic test

References

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