Splice-site Variants as Promising Novel Biomarkers in Clear Cell Renal Cell Carcinoma

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Background

Splice-site variants (SV) are DNA mutations that ultimately result in abnormal mRNA splicing and a dramatically altered protein-coding sequence. SVs in clear cell renal cell carcinoma (ccRCC) have been largely unexplored. Our primary objective was to identify the most prevalent SVs in ccRCC, determine their prevalence in other malignancies, and analyze associations with clinical outcomes.

Methods

Specific SVs were identified in RCC cell lines from the Broad Institute Cancer Cell Line Encyclopedia (CCLE). Patients with a diagnosis of ccRCC were then identified in the Moffitt Cancer Center Total Cancer Care (TCC) cohort and RNA-seq data was utilized in a bioinformatics pipeline to identify the SVs among the TCC cohort. SVs identified in the TCC ccRCC cohort were then identified in TCC patients with non-ccRCC malignancies. The 10 most prevalent SVs in ccRCC were selected for analysis, using a minimum SV read count threshold of 10. SV read count and SV dominance (specific SV read count / total SV read count) were assessed as metrics. Kaplan-Meier analysis and log-rank testing were utilized to assess differences in overall survival (OS) and recurrence free survival (RFS) between groups, stratified by the top quartile.

Results

Gene	SV Location	ccRCC pts + (n=104)	Other TCC pts + (n=4,174)
OXR1	8:107593479-107691437	104 (100%)	289 (6.9%)
GAL3ST1	22:30953388-30956727	101 (97.1%)	67 (1.6%)
GAL3ST1	22:30953388-30960811	96 (92.3%)	59 (1.4%)
PDZD2	5:31995744-32000244	77 (74.0%)	321 (7.7%)
TMEM44	3:194309368-194313769	75 (72.1%)	1106 (26.5%)
MVK	12:110032708-110032832	73 (70.2%)	39 (0.9%)
RNASET2	6:167369679-167370715	67 (64.4%)	911 (21.8%)
ASTN2	9:119739064-119741597	61 (58.7%)	42 (1.0%)
MVK	12:110032624-110032832	53 (50.1%)	25 (0.6%)
EGFR	7:55259222-55259411	51 (49.0%)	107 (2.6%)
TNS3	7:47568745-47579130	51 (49.0%)	49 (1.2%)
ITGB6	2:161056589-161057646	42 (40.4%)	55 (1.3%)

Figure 1. Splice site variants identified with high prevalence in ccRCC, positivity defined as read count >= 10.

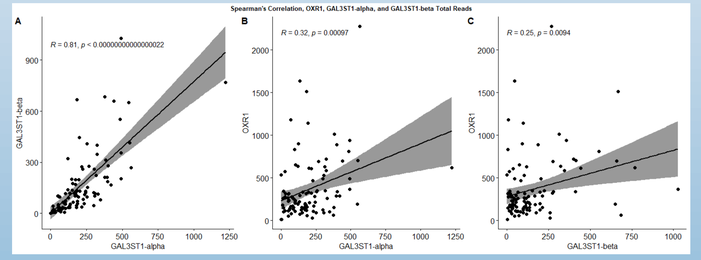

Figure 3. Spearman's correlations between the SVs OXR1, GAL3ST1-alpha, and GAL3ST1-beta. The two GAL3ST1 SVs had a strong positive read count correlation (R=0.81). OXR1 read count had a weak positive correlation with both GAL3ST1 subtypes.

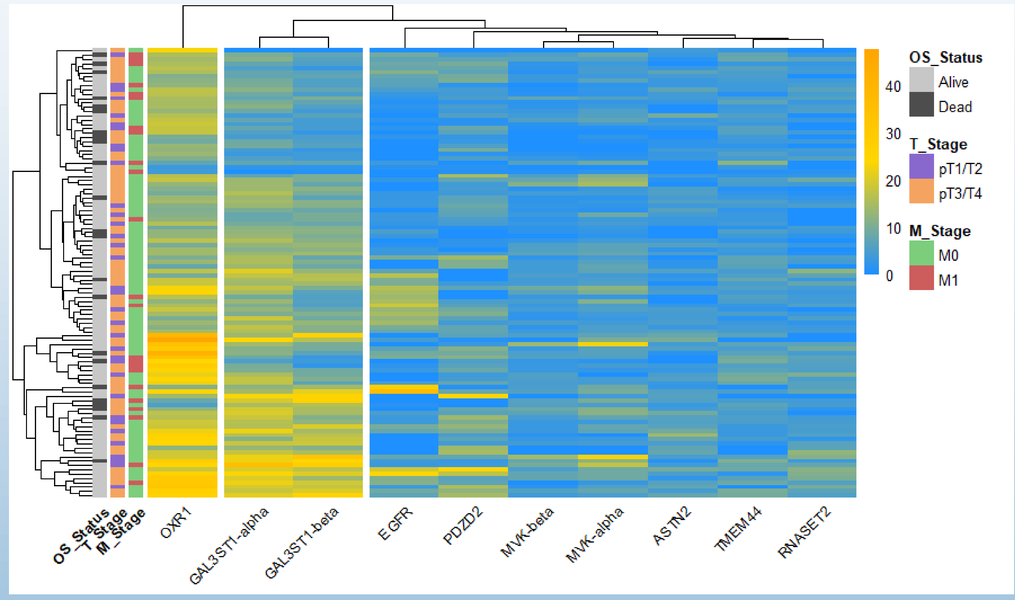

Figure 2. Heat map diagram of read counts for each of the 10 most prevalent splice site variants. Vitality status, T stage, and M stage presented as columns on the left of the diagram. Each row represents a patient.

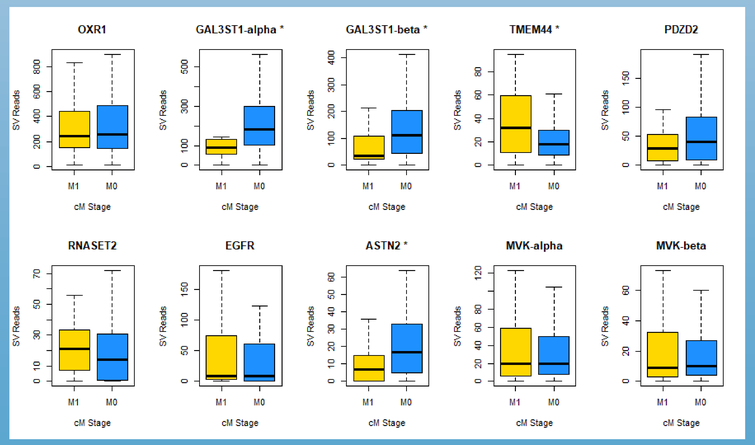

Figure 4. Boxplot diagrams associating SV read counts for each of the 10 most prevalent SVs in ccRCC, with cM stage. Asterix in plot title denotes Wilcoxon p <0.05.

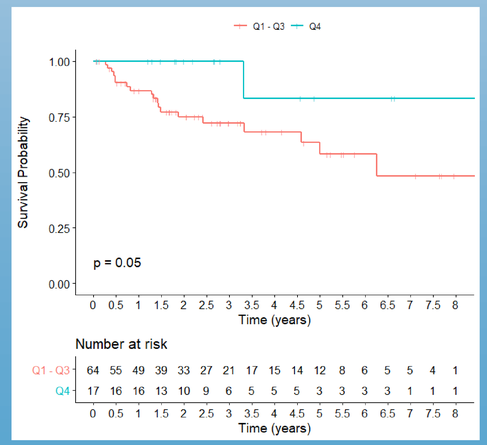

Figure 4. KM estimates for RFS, patients stratified by OXR1 SV read count dominance, Q4 versus other quartiles, log-rank p-value reported.

Conclusion

- We identified splice-site variants that are highly prevalent in ccRCC tumors and minimally prevalent in non-RCC malignancies.
- OXR1, GAL3ST1-alpha, and GAL3ST1-beta were the most prevalent SVs in ccRCC, and also had the highest read counts per positive patient.
- Low read counts of the SVs GAL3ST1-alpha, GAL3ST1-beta, and ASTN2 were associated with M1 disease at diagnosis.
- High OXR1 SV read count dominance was associated with improved recurrence free survival.