BITS :: Call for Abstracts 2019 - Oral communication

Type	Oral communication
Session	(Multi-)Omics Data Integration and Analysis
Title	Integrative analysis of multi-Omics data reveals that the dysregulation of splicing-related proteins in prostate cancer is controlled by FOXA1
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Motivation

The amount of high-throughput sequencing data has grown exponentially in the last decade and it represents a gold mine for cancer research being at the basis of the development of a personalized medicine. However, these high-volume data present an intrinsic heterogeneity, or inter-sample variability, that sets important challenges to their investigation. A considerable amount of high-throughput data is available for the study of prostate cancer (PC), the commonest male gender-specific cancer. Genomic characterisation of primary PC has uncovered several molecular subtypes, characterised by somatic alterations in genes encoding the transcription factors (TFs) FOXA1, ERG, AR, and HOXB13. Furthermore, genomic and transcriptional dysregulation of genes encoding RNA-binding proteins have been recently reported across several cancers, including PC. These proteins have pleiotropic roles in RNA metabolism, including pre-mRNA alternative splicing (AS). The altered expression of splicing factors was already proven to be a therapeutic vulnerability for cancers driven by the transcription factor MYC. Here we address the interplay between the dysregulation of transcriptional networks involving FOXA1, ERG, AR, and HOXB13 and the regulation of alternative splicing in PC.

Methods

RNA-sequencing data for 409 PC patient were collected from the The Cancer Genome Atlas (TCGA) consortium. Samples were stratified based on the high expression of the TFs and gene set analysis (GSA) was performed to identify the most altered biological processes. Cox proportional hazards models were used to perform a disease-free survival analysis of the cohort of patients based on the cumulative contribution of the expression of altered gene sets. Next, linear regression modelling was used to investigate the relationship between TFs and splicing-related genes (SRGs) expressions. Differentially expressed genes were detected using ‘DESeq2’ and ‘EdgeR’ software. A list of 62 ChIP-seq experiments of the TFs in LNCap and VCAP PC cell lines and TGCA ATAC-seq data of PC patients were integrated to study the regulation of the TFs on SRGs. TCGA splicing data available for a subset of PC patients were analysed in order to identify and characterize differential alternative splicing events. In vitro experimental validations of the results (i.e. RNA-seq, qRT-PCR, blotting) were performed on 4 PC cell lines (i.e. DU145, PC3, LNCaP, VCAP) by knockdown and over-expression of FOXA1.

Results

Using TCGA PC transcriptomic data we assessed the biological processes that are altered upon the high expression of FOXA1, ERG, AR, HOXB13 or MYC. By performing GSA, we detected a statistically significant association between the expression of all TFs and six different gene sets, including AS. To evaluate the impact of the dysregulation of the six gene sets on PC disease progression, we performed a survival analysis using Cox proportional hazards models. Of the six gene sets, we found that dysregulated

SRG expression showed the strongest association with disease recurrence. To determine the contribution of each TF to SRG regulation, we employed a linear regression modelling approach. We found that the overexpression of FOXA1 gave the best results in terms of determination coefficient when modelling SRG expression using only one TF gene. Additionally, after including FOXA1 expression level in the survival model we obtained the best results in terms of predictive power. Collectively, these findings suggest that the expression of FOXA1 shows the strongest association with the SRG modulation in PC. We next performed differential expression analysis and identified 76 SRGs that were significantly dysregulated upon the high expression of FOXA1. We next determined whether SRGs showed evidences of active binding of the TFs. Combining ChIP-seq and ATAC-seq data we found enrichment of FOXA1 binding sites within the promoter region of SRGs. These results suggest that FOXA1 might directly control the expression of SRGs in PC. We confirmed these results in vitro by knockdown and over-expression of FOXA1 in four PC cell lines. Furthermore, we demonstrated the persistence of a FOXA1-SRG association in lethal treatment-relapsed castration-resistant PC patients. We then assessed the outcome of FOXA1 dysregulation of SRGs on alternative splicing events and we showed a significant association between FOXA1 expression and a reduced variability of spliced isoforms. Finally, we validated our findings using RNA-seq performed on PC cell lines upon FOXA1 silencing. Analogous to MYC-driven cancers, our data demonstrate that the oncogenic TF FOXA1 controls the expression of SRGs, which is associated with poor PC patient outcomes. These findings implicate the possible therapeutic targeting of SRGs in FOXA1-overexpressing PC patients.

Info
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Availability	-

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