STAG2 role in gene regulation in muscle invasive bladder cancer (MIBC)

Zara Kazmierczak, Lanni Aquila, Nithya Krishnan, Swathi Ramakrishnan, Jianmin Wang, Eduardo Cortes Gomez, Anna Woloszynska-Read

Background:
Stromal Antigen 2 (STAG2) is one of the major subunits comprising the cohesin complex and is the most frequently mutated cohesin component in muscle invasive bladder cancer (MIBC). Independent of its role in chromatid segregation, STAG2 is thought to have a role in gene regulation. Based on evidence that STAG2 binding sites correspond to tissue-specific enhancers, we hypothesize that STAG2 acts as a transcription factor. Our objective is to investigate how STAG2 impacts BC biology through gene regulation and how this knowledge can be utilized in therapies.

Methods:
Whole and targeted exome sequencing revealed the presence of STAG2 functional mutations in over 10% of patients, with tumor immunochemistry confirming loss of STAG2 protein expression in mutated tumors. To study the impact of STAG2 loss on gene regulation in MIBC, we stably knocked down STAG2 in T24 cells via shRNA. Integrated analysis of ChIP (WT STAG2) and RNA sequencing (upon STAG2 knockdown) using Cistrome algorithm allowed us to identify STAG2 target genes. To map genome-wide chromatin accessibility upon loss of STAG2, we utilized ATAC sequencing. We used integrated analysis of increased accessibility (ATACseq) and gene expression (RNAseq) in T24 STAG2 wild type and knockdown cells to identify specific genomic regions of STAG2 transcriptional activity.

Results:
We observed that patients (n=330) with no STAG2 expression had prolonged median overall (34 vs. 24.5 months, p=.049) and progression free survival (23 vs. 13.5 months, p=.016) compared to STAG2 positive tumors. Integration of ChIP-seq and RNA-seq data identified epithelial-mesenchymal transition genes, such as MMP2, MMP9, SLUG, and SNAIL, targeted by STAG2. Cistrome with GeneHancer overlaps of STAG2 targets indicated 2811 potential enhancers regulated by STAG2. Integrated analysis of RNAseq and ATACseq data showed a decrease in chromatin accessibility upon knockdown of STAG2. shRNA-induced loss of STAG2 in T24 led to reduced chromatin accessibility. This correlated with increased expression of genes involving sister chromosomes resolution, cell cycles, rRNA modification and reduced expression of genes involving interferon signaling pathway and cellular matrix organization.

Conclusions:
Our results support the hypothesis that STAG2 promotes MIBC progression through transcriptional regulation and changes in chromatin accessibility. Our ongoing work will focus on functional validation of newly identified STAG2-regulated pathways and how STAG2-driven biology in bladder cancer can be exploited for new interventions.