Title: SPARC: A Bladder Cancer Tumor-Suppressor via Targeting Metabolic Programming

Background:

Bladder cancer (BC) has limited treatments and low survival in advanced disease. We identified Secreted Protein Acidic and Rich in Cysteine (SPARC) as a tumor suppressor in BC whose expression decreases in advanced disease. We sought to elucidate SPARC's role in regulating metabolic programming in BC and identify pathways that may serve as biomarkers and treatment targets.

Methods:

We used human BC cell lines UMUC3, T24, T24T. We assessed SPARC's effect on bioenergetics by knocking it down and treating with exogenous SPARC. We profiled transcriptomes of T24, T24T, and T24 SPARC-knockout to identify aggressive phenotype signatures using Gene Signature Enrichment Analysis. We compared signatures with survival data from BC cohorts from the Gene Expression Omnibus and The Cancer Genome Atlas. To determine SPARC's effect on several pathways, we performed in vitro mechanistic studies.

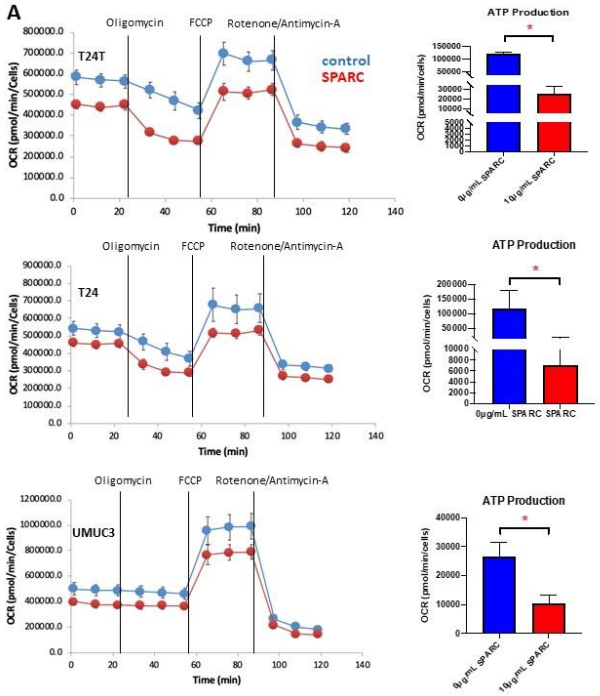
Results:

SPARC inhibited ATP production, basal and maximal respiration, and spare respiratory capacity in BC cells. The effect was greater on T24T cells than T24 cells. Depleting SPARC in T24 cells restored an aggressive phenotype, increased ATP production and mitochondrial respiration. Integrated transcriptomic profiling indicated SPARC loss is associated with enriched mitochondrial metabolic pathways. SPARC loss is also associated with oncogenic signatures. In patient samples, SPARC expression negatively correlated with enzymes in metabolic pathways associated with lower survival.

Conclusion:

Our data reveals a novel function of SPARC: inhibiting mitochondrial bioenergetics and ATP production that fuel growth and invasion in BC cells. Loss of SPARC in BC cells is associated with enriched oncogenic signatures. SPARC loss is linked to activation of pathways for proliferation, invasiveness, and metabolic programming of BC cells. This programming supports increased energy production to fill demands for malignant tumors. The SPARC-treated metabolic profile reveals BC cell vulnerabilities suggestive of a promising therapeutic target.

Figure 1:



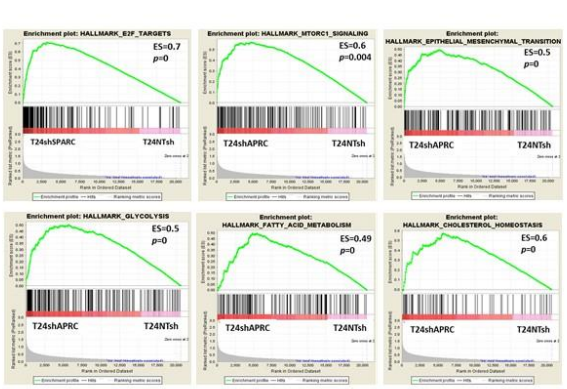
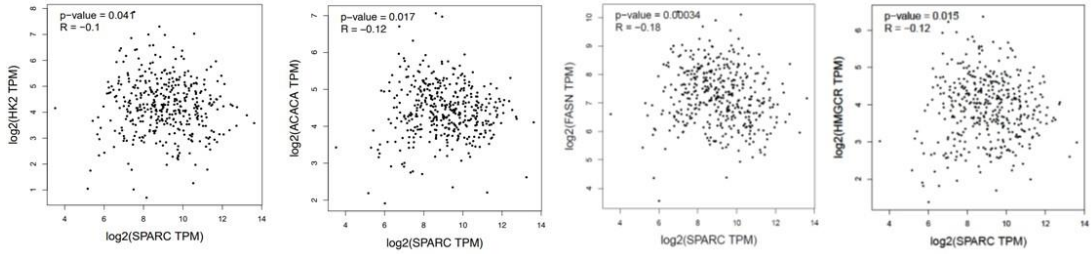


Figure 1: A. SPARC inhibits bladder cancer bioenergetics and ATP production. Seahorse tracing of mitochondrial oxygen consumption showing inhibition of basal, maximal and spare respiratory capacity after treating cells with exogenous SPARC. Bars represent mean ±SEM of ATP production in cells treated with SPARC. **p*<0.05, Students' *t*-test. **B. Gene Set Enrichment Analysis (GSEA) of top ranked significant genes in T24 cells depleted of SPARC (T24shSPARC) compared to controls (T24NTsh)** showing enrichment of signatures associated with survival, invasiveness and metabolic programming.

Figure 2:



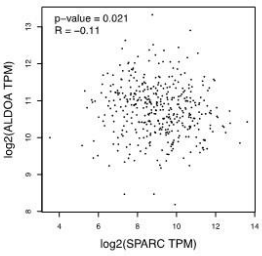


Figure 2: Gene Expression Profile Interactive Analysis (GEPIA) showing Pearson correlation between the expression of SPARC transcripts with the key enzymes involved in glycolysis, fatty acid and cholesterol metabolism *HK2*: hexokinase 2; *ACACA*: acetyl CoA carboxylase 1; *FASN*: fatty acid synthase; *HMGCR:* 3-Hydroxy-3-Methylglutaryl-CoA Reductase; and *ALDOA:* aldolase.