Novogene

RIP-seq and mRNA-seq

Client Publication in *Genome Biology*

The RNA binding protein SORBS2 suppresses metastatic colonization of ovarian cancer by stabilizing tumorsuppressive immunomodulatory transcripts

Background

Ovarian cancer constitutes one of the most lethal gynecologic malignancies. In this study, the RNA-binding proteins (RBPs) sorbin and SH3 domain containing 2 (SORBS2) is identified as a potent suppressor of ovarian cancer metastatic colonization. Mechanistic studies show that SORBS2 binds the 3′-untranslated regions (UTRs) of WFDC1 (WAP four-disulfide core domain 1) and IL-17D (Interleukin-17D), two secreted molecules that are shown to act as metastasis suppressors. By enhancing the stability of these gene transcripts, SORBS2 suppresses ovarian cancer invasiveness and affects monocyte to myeloid-derived suppressor cell and M2-like macrophage polarization, eliciting a tumor-suppressive immune microenvironment.

Research Pipeline

- **Samples:** A2780s ovarian cancer cell lines
- **Library Preparation:** RNA immunoprecipitation sequencing (RIP-seq) library and RNA-seq library
- **Sequencing Strategy:** Illumina HiSeq, PE150
- **Bioinformatics Analysis:** RIP-seq standard analysis and Gene expression analysis

Research Results

1 Integrated analysis identifies SORBS2 as a key RBP that suppresses ovarian cancer metastasis and its expression is associated with clinical outcome of ovarian cancer patients

Through cross-referencing a list of RBPs in the published literature, public oncogenomic data analysis, and searching in the cBioPortal TCGA ovarian cancer dataset, 4 genes were identified and showed deletion in more than 5% of TCGA ovarian cancer samples (Fig. 1a, b). Only SORBS2 and MEX3D gene knock-down significantly increased the metastatic colonization capacity of ovarian cancer (Fig. 1c). Only SORBS2 was significantly correlated with overall survival of ovarian cancer patients in the Australian Ovarian Cancer Study (AOCS) dataset (GSE9891)(Fig. 1d).

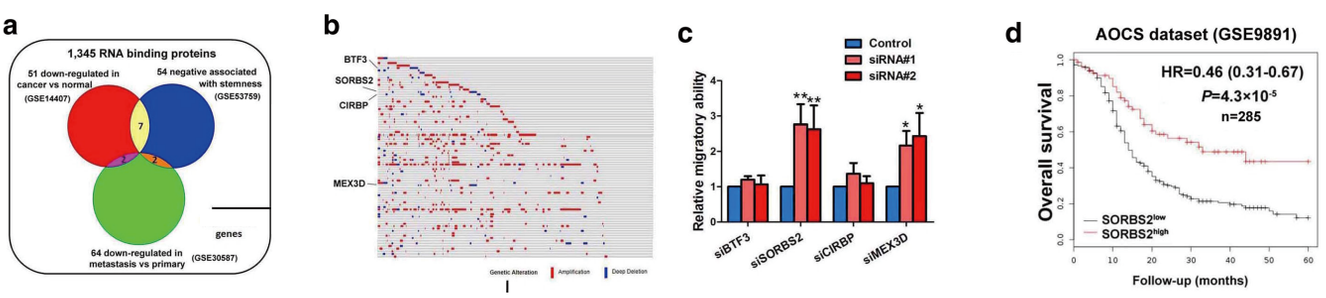

Figure 1. Outline of the screening strategy identifying the RBP SORBS2 as a key suppressor of metastatic colonization of ovarian cancer.

② WFDC1 and IL-17D mRNAs are bound by SORBS2 and destabilized by SORBS2 depletion

RIP sequencing was performed to identify endogenous RNA targets of SORBS2 in ovarian cancer cells (Fig. 2a). Relative transcript levels in treated SORBS2-depleted or control A2780s ovarian cancer cells were determined through transcriptomic sequencing, revealing a set of transcripts whose stability was deregulated upon SORBS2 loss. A proportion of transcripts bound by SORBS2 was found to overlap with the group of transcripts destabilized by SORBS2 knockdown compared with transcripts with no SORBS2-dependent changes in stability (Fig. 2b). Among the 91 gene transcripts potentially bound and stabilized by SORBS2, seven transcripts encoding secreted proteins attracted our attention since we focus on potential interactions between ovarian cancer cells and the tumor microenvironment during the metastatic process (Fig. 2c, d). WFDC1 and IL-17D exhibited reduced steady-state levels upon SORBS2 knockdown in both cell lines.

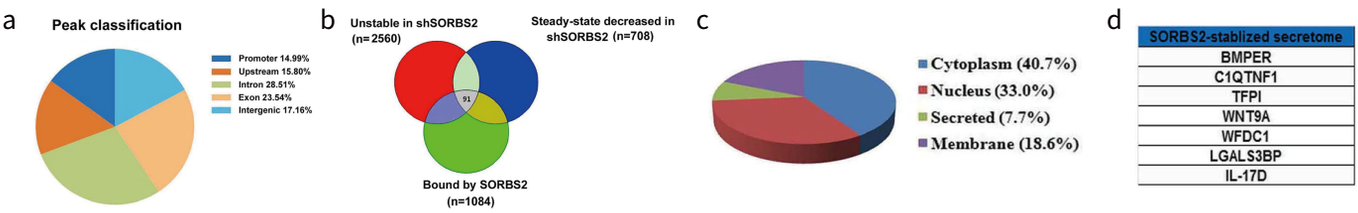

Figure 2. SORBS2 depletion affects the stability of transcripts directly bound by SORBS2.

③ SORBS2 depletion-induced secretome alterations are associated with monocyte to MDSC and M2-like macrophage polarization

RNA sequencing (RNA-seq) data from TCGA was used in bioinformatic co-expression analysis focusing on immune cell markers and cytokines. SORBS2 expression showed a significant negative correlation with the expression of 11 M2 myeloid cell markers and cytokines associated with their expansion (Fig. 3a). The similar significant negative correlation was also shown with combined SORBS2/WFDC1 (Fig. 3b) and SORBS2/IL-17D levels (Fig. 3c), respectively, constituting the tumor-suppressive immune microenvironment.

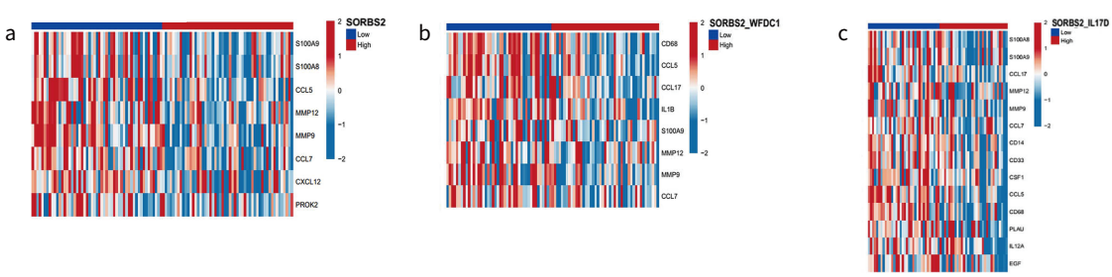

Figure 3. WFDC1 and IL-17D correlate with a tumor-suppressive immune profile in ovarian cancer patients.

Conclusion

This study takes advantage of the combination of RNA-seq and RIP-seq technology to illustrate a novel post-transcriptional network that links cancer progression and immunomodulation within the tumor microenvironment through SORBS2-mediated transcript stabilization.

Reference

Zhao L, Wang W, Huang S, *et al.* The RNA binding protein SORBS2 suppresses metastatic colonization of ovarian cancer by stabilizing tumor-suppressive immunomodulatory transcripts[J]. *Genome Biology,* 2018, 19(1): 35-35