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SPARCL1 suppresses metastasis in prostate cancer

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ABSTRACT

Purpose: Metastasis, the main cause of death from cancer, remains poorly understood at the molecular level.

Experimental design: Based on a pattern of reduced expression in human prostate cancer tissues and tumor cell lines, a candidate suppressor gene (SPARCL1) was identified. We used *in vitro* approaches to determine whether overexpression of SPARCL1 affects cell growth, migration, and invasiveness. We then employed xenograft mouse models to analyze the impact of SPARCL1 on prostate cancer cell growth and metastasis *in vivo*.

Abbreviations: CaP, cancer of the prostate gland; H.E., hematoxylin and eosin; IC, Intracardiac; IHC, Immunohistochemistry; IVIS, *in vivo* Imaging System; OX, orthotopic xenografting; PC3-Luc, the bioluminescent human prostate carcinoma cell line; PC3-luc/EV, GFP-positive PC3-Luc cells expressing empty control vector; PC3-luc/SPARCL1, GFP-positive PC3-Luc cells overexpressing SPARCL1; SCID, Severe combined immunodeficient; SPARCL1, secreted protein acidic and rich in cysteine-like 1.

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Meta-analysis
Metastasis
SPARCL1 function *in vivo*

Results: SPARCL1 expression did not inhibit tumor cell proliferation *in vitro*. By contrast, SPARCL1 did suppress tumor cell migration and invasiveness *in vitro* and tumor metastatic growth *in vivo*, conferring improved survival in xenograft mouse models.
Conclusions: We present the first *in vivo* data suggesting that SPARCL1 suppresses metastasis of prostate cancer.

1. Introduction

In men, cancer of the prostate gland (CaP) is the most commonly diagnosed non-cutaneous malignancy, accounting for 29% of all cancer cases and the second most common cause of death by cancer in the USA. In 2012, an estimated 241,740 men were diagnosed with CaP and 28,170 men died of CaP (Siegel et al., 2012; Jemal et al., 2010). The majority of cancer-associated deaths and essentially all CaP deaths are due to metastases rather than primary tumor burden (Gupta and Massague, 2006). Thus, decreasing mortality of CaP depends on understanding the biology that underlies metastasis such as identification of genes involved in cancer metastasis that would benefit the design of more effective clinical intervention strategies. There is a wealth of evidence indicating that the acquisition of malignant progression and aggressive traits of cancer can be promoted or inhibited by a set of functional genes known as metastasis-regulatory genes in various cancers (Cher et al., 1999). These can be broadly categorized as pro-metastasis or metastasis-suppressor genes. Pro-metastasis genes drive conversion from non-metastatic to metastatic cells (Seraj et al., 2000). Metastasis-suppressor genes suppress the formation of metastases without affecting primary tumor growth (Kauffman et al., 2003), a characteristic that distinguishes them from tumor-suppressor genes.

To identify candidate metastasis-regulatory genes in CaP, a common and straightforward method is to identify a list of differentially expressed genes (expression signature) from analysis of transcriptional profiles of CaP correlated with poor prognosis. However, the single study-based signature is often underpowered, truncated, and low quality. These limitations can be overcome by combining related but independent studies into a meta-analysis for larger sample size and lower false discovery rate. There are a limited number of published CaP gene-expression studies having clinical survival outcome data for meta-analysis. We previously used a robust meta-analysis of gene expression profiles from hundreds of breast cancer datasets (Yi et al., 2007; Wu et al., 2009; Qiu et al., 2013). Using this approach, we discovered a novel and conserved gene expression signature predictive of metastasis risk in multiple cancers (breast, lung, and prostate cancer) (Qiu et al., 2013). We hypothesized that this expression signature is enriched for genes that are mechanistically involved with cancer metastasis including CaP. We tested this idea for a candidate gene, secreted protein acidic and rich in cysteine-like 1 (SPARCL1).

There are sporadic data illustrating down-regulation of SPARCL1 in lung (Bendik et al., 1998), colorectal (Yu et al., 2011), urinary bladder (Zaravinos et al., 2011), pancreatic (Esposito et al., 2007), and prostate cancers (Taylor et al., 2010; Chandran et al., 2007; Yu et al., 2004; Dhanasekaran et al., 2001; Bendik et al., 1998; Nelson et al., 1998; Hurley et al., 2012). Recombinant SPARCL1 inhibited spreading and adhesion of bovine aortic endothelial cells (Brekken et al., 2004) and endothelial cells on fibronectin substrates *in vitro* (Girard and Springer, 1996). When its function was assessed using cancer cell lines, SPARCL1 inhibited pancreatic (Esposito et al., 2007) and prostate cancer cell migration and invasion *in vitro* but did not restrict the growth of prostate cancer cells (Hurley et al., 2012), suggesting that SPARCL1 is a potential suppressor of metastatic progression in prostate cancer. However, all previous results on SPARCL1 in CaP were derived from *in vitro* studies and clinical correlations. No *in vivo* data have been published to determine whether SPARCL1 contributes to CaP metastasis. Experiments, using a colon cancer cell line overexpressing SPARCL1 and a complementary model, suggested that SPARCL1 could reduce cell proliferation, anchorage-independent growth, and invasion *in vitro* and significantly inhibited orthotopic tumor growth *in vivo*. On this basis, Hu et al. concluded that SPARCL1 functions as a tumor suppressor in colon cancer (Hu et al., 2012). The question of whether SPARCL1 can suppress metastasis in CaP *in vivo* has not previously been addressed.

Consistent with previous studies, we found that SPARCL1 was down-regulated among human prostate tissue specimens and cell lines representing various levels of tumorigenicity and metastatic tendencies. However, we show here, in a prostate cancer model, that SPARCL1 does not inhibit tumor cell growth *in vitro* but does suppress tumor metastasis *in vivo*. Overexpression of SPARCL1 decreased the metastatic potential of human CaP (PC3) cells in both *in vitro* functional assays and *in vivo* experimental metastasis models. Specifically, SPARCL1 expression significantly inhibited tumor cell invasiveness and migration *in vitro* and capacity to metastasize to distant organs *in vivo*. These observations suggest that SPARCL1 can suppress metastasis in human CaP.

2. Materials and methods

2.1. Meta-analysis of human cancer profiles

The methods used for signature extraction, signature database development, and EXALT analysis were previously reported (Yi et al., 2007; Wu et al., 2009). Iterative EXALT analysis for identification of the 50-gene expression signature and its association with CaP metastasis has been described elsewhere (Qiu et al., 2013).