ORIGINAL RESEARCH

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Clinical relevance of semaphorin-3F in patients with prostate cancer

Abstract

Purpose: To identify prognosis predictors for patients with prostate cancer (PCa).

Methods: Four independent PCa microarray datasets (GSE32448, GSE16560, GSE79957 and GSE17951) were reanalyzed to characterize the expression of semaphorin-3F (SEMA3F) gene between PCa patients and normal prostate tissues and the correlation between SEMA3F expression and the age, tumor/nodes/metastasis (TNM) staging, Gleason Grade Group, prostate-specific antigen level and overall survival of PCa patients. Gene set enrichment analysis was applied to investigate the potential relevant mechanisms regarding the expression of SEMA3F and the proliferation of PCa cells.

Results: The level of SEMA3F was significantly higher in normal prostate tissues compared with that in PCa cells ($P < 0.0001$). Prostate cancer patients with higher expression of SEMA3F were associated with better TNM staging, Gleason Grade Group and overall survival than those with lower expression SEMA3F cohort. The result of gene set enrichment analysis indicated that SEMA3F might inhibit the proliferation of PCa cells through biological processes involving G2-mitosis checkpoint, E2F target and mitotic spindle.

Conclusion: Data suggest that SEMA3F might be a tumor suppressor of PCa and a protective factor for patient with PCa.

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Prostate cancer (PCa) is the second most commonly diagnosed cancer and the fifth leading cause of cancer death in men [1,2]. An estimated 1.1 million new PCa cases and 307 thousand PCa deaths occurred in 2012 worldwide [3]. Since the advent of early detection efforts, such as prostate-specific antigen (PSA) screening, and increased public awareness, PCa is frequently diagnosed in its initial stages. Prostate-specific antigen is a biomarker for the diagnosis, monitoring and risk prediction of PCa. Although PSA screening has aided in the detection of PCa in millions of men across the world, which has resulted in an improvement in 5-year survival of PCa patients [4-6], complications of PCa, such as bone metastases and skeletal-related events, remain a major clinical concern.

Androgen deprivation therapy (ADT), including gonadotropin-releasing hormone (GnRH) agonists/antagonists and surgical castration, is a longstanding treatment for PCa. Achieving castration levels of testosterone with ADT is a time-limited intervention, and, most PCa patients on ADT inevitably become “castration-resistant”. Thus, identification of biomarkers that could predict the progression and survival of PCa patients may be of clinical importance [7-9].

Semaphorin-3F (SEMA3F) was originally isolated from a recurrent 3p21.3 homozygous deletion in small-cell lung cancer cells suggesting that it might be a tumor suppressor gene [10]. Semaphorin-3F belongs to a large family of secreted, transmembrane and membrane-associated semaphorin proteins that is characterized by a conserved, cysteine-rich, 500 amino-acid “sema” domain. Semaphorin-3F is a class 3 semaphorin, as it is secreted and contains an immunoglobulin-type domain. Included among proteins with sema-domains are plexins and the oncogenic Met and Ron receptor tyrosine kinases [11]. Previous studies demonstrated that SEMA3F is down-regulated in lung, liver, breast and ovarian cancer and in melanoma. It was previously reported that SEMA3F expression was significantly reduced in human colorectal cancer tissues and cell lines and that SEMA3F inhibits the growth and metastasis of colorectal cancer cells [12]. Over-expression of SEMA3F in colorectal cancer cells results in reduced cell proliferation, adhesion and migration associated with reduced expression of integrin $\alpha\text{v}\beta\text{3}$ [13]. However, there have been no reports concerning the prognosis value of SEMA3F in patients with PCa.

In the present study, we showed that SEMA3F was downregulated in PCa cells. Prostate cancer patients in the SEMA3F higher expression group had better Gleason Grade Group and tumor/nodes/metastasis (TNM) staging compared with patients in the corresponding SEMA3F

lower expression group. Moreover, survival analysis of SEMA3F suggested that higher expression SEMA3F predicted better overall survival of PCa patients.

Materials and methods

Data collection

Four independent PCa microarray datasets (GSE32448 [17], GSE16560 [14], GSE79957 [18] and GSE17951 [15,16]) were obtained from Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>). The GSE32448 dataset, which included 40 PCa samples and 40 normal prostate tissues, was used to compare the expression of SEMA3F in PCa tissue and normal prostate tissue. The GSE16560 dataset, which included 281 cases of PCa from the population-based Swedish-Watchful Waiting cohort and included information on patients' ages, the Gleason Grade Groups and survival of the PCa patients, was used to compare the patients' ages, Gleason Grade Groups and overall survival of PCa patients in the SEMA3F low and high expression groups. The GSE79557 dataset, which included 260 PCa samples from 260 patients and included clinical information on TNM staging and preoperative PSA, was used to compare the distribution of these factors in the SEMA3F low and high expression groups. The GSE17951 dataset, which included 154 PCa samples, was used to perform gene set enrichment analysis (GSEA) [19].

Data analysis

The expression of SEMA3F (the probe ID is 206832_s_at in GSE32448) in PCa and normal prostate tissue is presented as mean $\pm$ standard deviation (SD). The PCa samples were divided into SEMA3F low and high expression groups based on the medians of SEMA3F expression (0.036 and 0.041, respectively) in GSE16560 (the corresponding probe ID was DAP3_0864) and GSE79957 (the corresponding probe ID was 2622547). Student's t-test was used to compare the SEMA3F expression in PCa group and normal prostate tissue group, and also used to analyze the difference of PCa patients' age and PSA levels between the SEMA3F high and low expression groups. The χ^2 analysis was used to characterize the relationship between the expression of SEMA3F and clinical features (including age, TNM staging, Gleason Grade Group and PSA) of PCa patients. Log-rank based survival analysis was conducted to characterize the expression of SEMA3F and the overall survival of PCa patients. A value of $P < 0.05$ was considered statistically significant for Student's t-test, χ^2 test and survival analysis. Gene set enrichment analysis was conducted to infer the potentially relevant mechanism involved in the regulation of

SEMA3F on PCa cells and h.all.v5.2.symbols.gmt was used as a reference gene set. Nominal p value <0.05 and false discovery rate less than 25% was defined as statistical significance.

Results

Semaphorin-3F was downregulated in PCa cell

As shown in Figure 1, the expression of SEMA3F was downregulated in PCa samples compared with that in normal prostate tissues ($P<0.0001$).

Relationship between semaphorin-3F expression and clinicopathological features

As shown in Tables 1 and 2, there was no significant difference regarding the age and PSA of PCa patients between SEMA3F low and high expression groups. In Table 2, patients with higher SEMA3F expression had better Gleason Grade Group and TNM staging compared with patients with lower SEMA3F expression ($P<0.0001$ and 0.04, respectively). These results suggested that SEMA3F might inhibit the progression of PCa.

Higher expression of SEMA3F predicted better overall survival of PCa patients

To confirm the impact of SEMA3F expression on the survival of PCa patients, we conducted survival analysis of PCa patients with SEMA3F high expression and PCa patients with SEMA3F low expression using PCa microarray GSE16560 [14]. As shown in Figure 2, PCa with SEMA3F high expression had better overall survival compared with those with SEMA3F low expression (HR=1.796, 95% CI: 1.352-2.386, $P<0.0001$), suggesting that PCa patients with SEMA3F expression have longer overall survival than those with SEMA3F low expression.

Gene set enrichment analysis of PCa samples with SEMA3F low expression

To characterize the potentially relevant mechanism involved in the SEMA3F impact on the proliferation of PCa cells, PCa samples from another independent PCa microarray study (GSE17951) [15,16] were divided into SEMA3F high and low expression groups based on the median of SEMA3F, and GSEA was performed. As shown in Table 3, PCa samples with SEMA3F low expression were enriched in gene sets associated with G2M checkpoint, E2F target and mitotic spindle, suggesting that SEMA3F might suppress the proliferation of PCa cell through regulating cell cycle or E2F signaling.

FIGURE 1. Expression of SEMA3F was downregulated in prostate cancer compared with normal prostate tissue ($P<0.0001$)

FIGURE 2. Prostate cancer samples with SEMA3F high expression had better overall survival compared with SEMA3F low expression

TABLE 1. Comparison of patient's age and prostate-specific antigen between semaphorin-3F high and low expression groups

	SEMA3F low expression (n=140)	SEMA3F high expression (n=141)	t	P	Data source
Age (year)	69.74±3.74	71.02±5.38	-1.37	>0.05	GSE16560
Prostate-specific antigen	12.55±4.55	11.45±5.17	0.797	>0.05	GSE79557

Data are expressed as mean ± standard deviation

TABLE 2. Relationship between semaphorin-3F expression and the clinicopathological features in PCA patients

Clinicopathological feature	SEMAF low expression	SEMA3F high expression	χ ²	P value	Data source
Age (year)					
≤70	37	46	1.296	0.255	GSE16560
70	103	95			
Gleason Grade Groups					
GG1 (Gleason 6)	25	60	21.254	P<0.0001	GSE16560
≥GG2 (3+4)	115	81			
TNM staging					
T2	25	41	4.228	0.04	GSE79957
T3	105	89			
Prostate-specific antigen levels (ng/ml)					
≤9.5	68	65	0.139	0.71	GSE79557
>9.5	62	65			

TABLE 3. Gene set enriched in prostate cancer samples with semaphorin-3F low expression

Name	Size (kbase pairs)	Enrichment score	Normalized enrichment score	Nominal P value	False discovery rate q-value
G2M_CHECKPOINT	178	0.63957	1.855053	0.012097	0.094816
E2F_TARGETS	173	0.620747	1.844577	0.016194	0.052077
MITOTIC_SPINDLE	185	0.481782	1.742247	0.02521	0.089949

Discussion

As mentioned above, because of the high incidence of PCa and inevitable disease relapse or drug-resistance in patients with PCa, the prognosis of PCa patient remains poor, and exploitation of targeted therapy of PCa patients is urgent. In the present studies, we reanalyzed four independent PCa microarray studies (GSE32448 [17], GSE16560 [14], GSE79957 [18] and GSE17951 [15,16]), demonstrating that SEMA3F was downregulated in PCa cells. The PCa patients in SEMA3F high expression group were better in terms of the Gleason Grade Group, TNM staging and overall survival than those in SEMA3F low expression.

Semaphorin-3F is a member of the class 3 semaphorin family, originally characterized in axonal guidance [11,20]. Previous studies have demonstrated that SEMA3F played a role as a tumor suppressor in prostate and other cancers. Xie Wen et al. found that the degree of PCa aggressiveness was inversely associated with the AKAP12 and SEMA3F expression [21]. Bielenberg et al. demonstrated that SEMA3F was markedly downregulated in highly metastatic human cell lines in vitro and in vivo, and then transfected metastatic human melanoma cells with SEMA3F and implanted the melanoma cells into mice. The resultant tumors did not metastasize and the primary tumors resembled benign nevi, characterized by large areas of apoptosis, diminished vascularity, inhibition of hyperplasia in overlying epidermal cells and encapsulated tumor borders delineated by thick layers of fibroblasts and collagen matrix. These results suggested that SEMA3F induced a poorly vascularized, encapsulated, non-metastatic tumor phenotype [22]. Doçi et al. demonstrated that SEMA3F, widely lost during cancer progression, served as an anti-lymphangiogenic metastasis suppressor gene in head and neck squamous carcinoma [23]. Yang et al. proved that SEMA3F suppressed p53-induced pathways, which lead to axonal growth cone collapse in primary hippocampal neurons [24]. Potiron et al. demonstrated that SEMA3F inhibited the proliferation of lung cancer through negatively affecting ILK-ERK1/2 and AKT-STAT3 signaling, along with inhibition of HIF-1A and VEGF [10]. Shimizu et al. demonstrated that ABL2/ARG tyrosine kinase mediated SEMA3F-induced RhoA inactivation and cytoskeleton collapse in human glioma cells [25].

These published studies indicate that SEMA3F functioned as a tumor suppressor in a variety of human cancers through the negative regulation of cell proliferation, attachment, spreading and migration, either in vivo or in vitro. Our results also suggest that SEMA3F may acted as a tumor suppressor in PCa cells. Semaphorin-3F was downregulated in PCa cells, indicating that the loss of

SEMA3F might contribute to the pathogenesis of PCa. The TNM staging and Gleason Grade Group are often treated as indices of tumor progression in PCa, and a higher stage of TNM or higher Gleason Grade Group represents a worse prognosis in patients with PCa [26-29]. Our results showed that the stage of TNM and the Gleason Grade Group of PCa patients in the SEMA3F high expression group were lower compared with those in SEMA3F high expression group, suggesting that SEMA3F might inhibit the proliferation and progression of PCa. Finally, our results suggest that the prognosis of PCa patients with SEMA3F high expression was better than those with SEMA3F low expression, suggesting that SEMA3F might be a protective factor and could be used as a prognosis predictor for patients with PCa.

There was no significant difference in the PSA levels between patients with SEMA3F high and low expression. The PSA, or human kallikrein 3 (hK3), is a glycoprotein formed by the prostate gland [5,6]. As mentioned above, the introduction of the measurement of prostate specific antigen in the mid-eighties of last century represented a major innovation in the management of patients with PCa. However, the specificity of PSA was debatable. Biopsies tested positive in approximately 25% of patients with PSA in the range 20–10 µg/L. Patients with benign prostatic hyperplasia were often detected with elevated levels of PSA, and elevated levels of PSA are particularly found in patients with enlarged prostate volume. Moreover, the normal range for PSA increases with age. Thus, it would not be surprising that the levels of PSA were similar between PCa patients with SEMA3F high and low expression groups.

In conclusion, our results suggested that SEMA3F might be a tumor suppressor of PCa and a protective factor for patient with PCa.

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