Am J Cancer Res 2015;5(8):2498-2507
www.ajcr.us /ISSN:2156-6976/ajcr0008408

Original Article

Sprouty2 protein in prediction of post-treatment ascites in epithelial ovarian cancer treated with adjuvant carbotaxol chemotherapy

Samar Masoumi-Moghaddam[1], Afshin Amini[1], Ai-Qun Wei[2], Gregory Robertson[3], David L Morris[1]

[1]Department of Surgery, St George Hospital, The University of New South Wales, Kogarah, Sydney NSW 2217, Australia; [2]Department of Orthopedic Surgery, St George Hospital, The University of New South Wales, Kogarah, Sydney NSW 2217, Australia; [3]Department of Gynecology Oncology, St George Hospital, The University of New South Wales, Kogarah, Sydney NSW 2217, Australia

Received March 24, 2015; Accepted June 19, 2015; Epub July 15, 2015; Published August 1, 2015

Abstract: Ascites development and resistance to chemotherapy with carbotaxol are common clinical problems in epithelial ovarian cancer, partly due to the activation of MAPK/ERK signaling. Sprouty proteins are negative modulators of MAPK/ERK pathway, but their role in predicting resistance to carbotaxol chemotherapy and ascites development is unknown. In this study, we evaluated the expression of Sprouty protein isoforms by immunohistochemistry. The associations between the Sprouty expression and the clinicopathological features, including chemoresistance and the presence of ascites, were then explored. We found that the decreased expression of Spry2 was correlated with the post-treatment development of ascites and represented an independent predictor of this condition in carbotaxol-treated patients. However, no association was observed between the Sprouty expression and chemoresistance. In conclusion, our results suggest that Spry2 may be useful for patient follow-up and monitoring as it predicts the development of ascites in epithelial ovarian cancer cases treated with carbotaxol.

Keywords: Ascites, epithelial ovarian cancer, refractory disease, Sprouty1, Sprouty2, Sprouty4

Introduction

Accounting for around 3.6% of female cancers, epithelial ovarian cancer (EOC) is the seventh leading cancer in women and the second cause of gynecological cancer death worldwide [1]. Late diagnosis (due to unspecific clinical manifestation), recurrence and refractoriness are major contributors to the disease poor survival rates [2]. Ascites presents in at least one third of patients and may contribute to the spread of cancer to secondary sites [3]. Postoperative treatment with paclitaxel plus carboplatin (carbotaxol) is recommended for patients with early-stage disease with poor prognostic features [4]. For advanced-stage disease, the current standard first-line chemotherapy regimen involves intravenous administration of a platinum-based drug (cisplatin/carboplatin) with a taxane, usually paclitaxel, given 3 weekly for six cycles. Although up to 50% of patients achieve complete clinical and radiological remission with this chemotherapy regimen, 20-30% show no evidence of response. In addition, the disease recur in most patients with advanced EOC [5]. Lack of novel and specific sets of markers for diagnosis, clinical monitoring, prognosis and prediction of response to treatment is still considered as an unmet need to improve medical management of this disease. Thus, more investigations for identification of new markers are warranted. This may also result in the development of new treatment modalities for better management of this highly refractory recurrent disease.

Among the biological pathways being activated in cancer and involved in the development of chemoresistance is mitogen-activated protein kinase (MAPK) signaling [6]. Members of the Sprouty protein family, including Spry1, Spry2 and Spry4, are known as the downstream modulators of MAPK and thus largely contribute to

the regulation of the eukaryotic cells biology [7]. In agreement, different patterns of the Sprouty deregulation have been reported in a variety of cancers. To date, however, no studies have investigated the possible association of the Sprouty proteins with the development of ascites and response to carbotaxol chemotherapy in EOC. This study investigates the likely correlation between the Sprouty expression in EOC and the afore-mentioned clinicopathological characteristics which could yield a better understanding of the role of Sprouty in this cancer. It may also lay foundation for further assessment of Sprouty as a protein family with potential application in diagnostic, therapeutic and prognostic approaches.

Patients and methods

Patient selection

A retrospective review of the clinical records of 480 patients with EOC from two specialized centers (St. George Hospital and St George Private Hospital, Sydney, Australia) was performed between January 2001 and December 2012. Institutional review board approval for this analysis was obtained from South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee. After obtaining informed consents, histologically-proven cases of primary EOC who had a complete follow-up history till June 2014 (end of the study) and were treated with the standard surgical procedure (staging laparotomy/cytoreductive surgery) plus adjuvant systemic chemotherapy (paclitaxel + carboplatin as formulated below) were included in this study.

Adjuvant chemotherapy regimen:

Paclitaxel (175 mg/m^2, iv over 3 hours) + carboplatin (total dose calculated by Calvert formula*, iv over 15-60 minutes) × 6 cycles.

*Total carboplatin dose (mg) = Target area under concentration vs time curve (AUC) × (GFR +25).

A confirmatory review of pathology was performed. Ovarian neoplasms were histologically classified according to The World Health Organization (WHO) classification system [8]. The final staging of the disease was determined on the basis of a combination of surgical and pathological findings in accord with the Federation of Gynecology and Obstetrics (FIGO) guidelines [9].

Immunohistochemistry

Five-micrometer sections were prepared from the paraffin blocks and floated onto positively charged slides. Immunostaining was performed as described previously [10]. Briefly, the sections were deparaffinized and microwaved in either 10 mM sodium citrate buffer (pH 6.0) (Sigma-Aldrich, USA) for Spry1 and Spry2 or 10 mM Tris base, 1 mM EDTA solution at pH 9.0 (Sigma-Aldrich, USA) for Spry4 for 20 min at 750 W for antigen retrieval. Thereafter, the samples were incubated with 3% hydrogen peroxide and DAKO blocking buffer, respectively. This was followed by the overnight incubation of samples at 4°C with primary antibodies (Abnoval, Taiwan) at dilutions of 1/500, 1/100 and 1/250 for Spry1, Spry2 and Spry4, respectively. Binding of the primary antibody was detected by incubating the samples with appropriate secondary antibody using EnVision Plus kit (DAKO) for 30 min and then with diaminobenzidine chromogen for 5 min. The sections were then rinsed, counterstained with hematoxylin, and mounted. Kidney, small bowel/testis and testis were included as positive controls for Spry1, Spry2 and Spry4, respectively. For negative controls, the same tissues as our positive controls for each antibody were used but the primary antibodies were replaced with the primary antibody diluents.

Evaluation of the staining

To evaluate the staining of the epithelial cells, we performed semi-quantitative scoring based on the method used by Kwabi-Addo et al [11]. This scoring method enables the determination of both the intensity of the immunosignal and the percentage of cells showing positive staining. The immunohistochemical expression was evaluated by at least two observers blinded to the patients outcome. Based on the intensity of the staining, samples were scored 0 (no staining), 1 (weak), 2 (moderate) or 3 (strong). As regards the percentage of the positive cells, samples were scored 0 (no positive cells), 1 (1-33%), 2 (34-66%) or 3 (67-100%). Finally, the overall staining scores ranging from 0 to 9 were calculated as follows: immunohistochemical

Table 1. Correlations of the expressions of Sprouty isoforms with clinicopathological characteristics of EOC patients

Parameter			No	Spry1	No	Spry2	Spry4
				p value*		*p* value*	*p* value*
Age (year)	≤50		16	**0.022**	15	0.811	0.329
	>50		84		84		
Menopause	Yes		92	0.124	91	0.599	0.459
	No		8		8		
Disease stage	Early (I-II)		14	**0.029**	14	**0.013**	0.614
	Advanced (III-IV)		86		85		
Tumor grade	I-II		23	**0.037**	22	**0.003**	0.530
	III		77		17		
Tumor subtype	Serous		81	0.647	80	0.216	0.094
	- High-grade		63		63		
	- Low-grade		18		17		
	Mucinous		2		2		
	Endometrioid		4		4		
	- High-grade		2		2		
	- Low-grade		2		2		
	Clear cell		5		5		
	Others		8		8		
Lymphovascular invasion	Yes		35	**0.042**	35	0.298	0.716
	No		25		25		
Lymph node involvement	Yes		38	0.511	38	0.053	0.112
	No		25		25		
Response to chemotherapy	No		21	0.321	21	0.250	0.944
	Yes	Recurrent	58	**0.001**	57	**<0.001**	0.197
		Non-recurrent	21		21		
Ascites	Yes		65	0.248	64	**0.028**	0.669
	No		35		35		
Residual tumor	None		48	0.762	47	0.888	0.111
	<1 cm		35		35		
	1-2 cm		0		0		
	>2 cm		17		17		

No: number of patients, Spry: Sprouty. *Statistically significant values (*p* value <0.05) are shown in bold.

score = [percentage of the positive cells] × [intensity of the staining].

Statistical analysis

All statistical analyses were conducted using the statistical package SPSS, version 22 (SPSS Inc., USA). The data were summarized using standard descriptive statistics and frequency tabulations. Spearman correlation coefficient testing was performed to evaluate the associations between the clinicopathological parameters, including age, menstrual status, tumor subtype, stage, grade, response to chemotherapy, recurrent disease, presence of ascites and the expressions of Sprouty isoforms. Using the Classification and Regression Tree (CART) algorithm, the binary cut-off points of the expression values were identified -which were near the median values- and the immunohistochemical scores obtained were accordingly classified as low (scores ≤3.5 for Spry1 and Spry2, and ≤6 for Spry4) or high (scores >3.5 for Spry1 and Spry2, and >6 for Spry4). Analysis of receiver operating characteristic (ROC) curves was performed to assess the validity of the cut-off points and also the sensitivity and specificity of the markers with significant predictive values. Univariate and multivariate logistic regression

Table 2. Correlations of the expressions of Sprouty isoforms with history of ascites in EOC patients

Parameter		No.	Spry1	No.	Spry2	Spry4
			p value*		*p* value*	*p* value*
Overall ascites	Yes	65	0.248	64	**0.028**	0.669
	No	35		35		
Ascites at diagnosis	Yes	54	0.302	53	0.504	0.883
	No	46		46		
Post-treatment ascites	Yes	42	0.100	41	**0.001**	0.466
	No	58		58		
Post-treatment only[a]	Yes	11	0.595	11	**0.007**	0.619
	No	35		35		
Pre-treatment only[b]	Yes	23	0.180	23	0.068	0.608
	No	31		30		
Pre- and post-treatment ascites[c]	Yes	31	0.151	30	0.263	0.593
	No	34		34		

No: number of patients, Spry: Sprouty. [a]: 46 patients with no ascites at diagnosis among which 11cases developed post-treatment ascites; [b]: 54 patients with ascites at diagnosis among which 23 cases did not develop ascites after treatment; [c]: 31 patients with ascites at both diagnosis and post-treatment out of 65 cases with ascites history. *Statistically significant values (*p* value <0.05) are shown in bold. Number of patients in each group might slightly differ for Spry2 and Spry4 due to the lack of available tissue for analysis.

analyses were conducted to ascertain the effects of Sprouty isoforms and other clinicopathological variables on the likelihood of the development of post-treatment ascites and chemotherapy refractory disease. For all statistical analyses, *p* values <0.05 were considered significant. Student t-test was used to compare the actual difference between two means.

Results

Of the 100 participants with EOC, 81% had tumors of serous subtype (63% high-grade and 18% low-grade), 4% had endometrioid tumor (2% high-grade and 2% low-grade), and 2% and 5% were identified as mucinous and clear cell subtypes, respectively. The median age of the entire cohort was 62.2 (range, 35.32-84.3) years. 92 patients were menopausal at the time of diagnosis. The mean expression scores of Spry1, Spry2 and Spry4 were 3.01±0.20 (range; 0-6), 2.76±0.14 (range; 0-6) and 5.38±0.24 (range; 0-9), respectively. Among the clinicopathological characteristics analyzed, recurrent disease (correlation coefficients, Spry1: -0.379; Spry2: -0.450), disease stage (correlation coefficients, Spry1: -0.218; Spry2: -0.248) and tumor grade (correlation coefficients, Spry1: -0.209; Spry2: -0.297) were significantly associated with the expressions Spry1 and Spry2 in an inverse manner. Moreover, there was a significant correlation between the Spry2 expression status and the history of ascites (*p* value: 0.028). A correlation between the Spry1 expression and age was also evident (*p* value: 0.022, correlation coefficient = 0.229). However, no association was found between Spry4 and the studied characteristics (**Table 1**).

Patients with ascites had lower Sprouty expression

Overall, 65% of patients had confirmed ascites either at the time of diagnosis or after treatment. In the subgroup with ascites at the time of diagnosis, the mean expression scores of Spry1, Spry2 and Spry4 were 2.70±0.27, 2.74±0.18 and 5.17±0.35, respectively, compared with 3.37±0.29, 2.80±0.21 and 5.63±0.33 in those without ascites at diagnosis. Although this subgroup showed lower expression levels of the three Sprouty isoforms than did the non-ascites group, the differences were not statistically significant (*p* values of 0.102, 0.81 and 0.355 for Spry1, Spry2 and Spry4, respectively). In the other subgroup with post-treatment ascites, mean expression scores of Spry1, Spry2 and Spry4 were 2.36±0.30, 2.34±0.20 and 4.98±0.39, respectively, as compared with 3.48±0.25, 3.07±0.182 and 5.67±0.31 in those without post-treatment ascites. The statistical comparison with the non-ascites control revealed significantly lower expressions of Spry1 (*p* values: 0.006) and Spry2 (*p* values: 0.010), in this subgroup. However, the decrease in the expression of Spry4 was not statistically significant (*p* values: 0.166).

Spry2 correlated with the presence of ascites in EOC patients

The clinical relevance of the Sprouty expression with regard to the development of ascites in EOC was initially evaluated through the analysis

Table 3. The predictive value of Spry1, Spry2 and Spry4 for response to chemotherapy and post-treatment ascites

Variables	Post-treatment ascites		Response to chemo# (Refractory)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariate				
Age (year) (≤50 vs. >50)	1.089 (0.370-3.203)	0.877	0.489 (0.102-2.343)	0.371
Menopause (no vs. yes)	1.421 (0.334-6.038)	0.634	1.281 (0.239-6.858)	0.773
Stage (early vs. late)	0.084 (0.011-0.674)	**0.020**	0.588 (0.121-2.857)	0.510
Tumor grade (I-II vs. III)	0.525 (0.194-1.420)	0.204	1.059 (0.341-3.290)	0.921
Tumor subtype (HG serous vs. LG serous vs. HG endometrioid vs. LG endometrioid vs. mucinous vs. clear cell vs. others)	0.450 (0.099-2.049)	0.302	0.189 (0.040-0.882)	**0.034**
Lymphovascular invasion (no vs. yes)	1.00 (0.351-2.851)	1.00	0.625 (0.184-2.125)	0.452
Lymph node involvement (no vs. yes)	0.484 (0.157-1.491)	0.206	0.439 (0.106-1.817)	0.256
Ascites at diagnosis (no vs. yes)	0.233 (0.098-0.554)	**0.001**	1.086 (0.414-2.847)	0.867
Residual tumor (no vs. <1 cm vs. 1-2 cm vs. >2 cm)	0.404 (0.130-1.252)	0.116	0.225 (0.067-0.761)	**0.016**
Refractory disease (no vs. yes)	0.153 (0.051-0.464)	**0.001**	N/A	N/A
Spry1 (high vs. low)	0.492 (0.211-1.147)	0.101	0.588 (0.206-1.675)	0.320
Spry2 (high vs. low)	0.236 (0.086-0.648)	**0.005**	0.480 (0.147-1.570)	0.225
Spry4 (high vs. low)	0.493 (0.220-1.104)	0.085	0.761 (0.290-1.996)	0.579
Multivariate				
Post-treatment ascites				
Stage (early vs. late)	0.150 (0.015-1.547)	0.111		
Ascites at diagnosis (no vs. yes)	0.193 (0.066-0.567)	**0.003**		
Refractory disease (no vs. yes)	0.098 (0.025-0.385)	**0.001**		
Spry2 (high vs. low)	0.256 (0.078-0.838)	**0.024**		
Response to chemo # (Refractory)				
Tumor subtype (HG serous vs. LG serous vs. HG endometrioid vs. LG endometrioid vs. mucinous vs. clear cell vs. others)	0.100 (0.019-0.534)	**0.007**		
Residual tumor (no vs. <1 cm vs. 1-2 cm vs. >2 cm)	0.140 (0.035-0.563)	**0.006**		

HR: hazard ratio; CI: confidence interval; #Chemotherapy with carboplatin and taxol. Statistically significant values (*p* value <0.05) are shown in bold.

Table 4. Sensitivity and specificity of Spry2 expression in prediction of the development of post-treatment ascites

Test	Post-treatment Ascites
	Spry2 ≤3.5
True positive	44
False positive	29
True negative	21
False negative	6
Sensitivity (95% CI)	88.00% (75.68%-95.44%)
Specificity (95% CI)	42.00% (28.19%-56.79%)
Positive predictive value (95% CI)	60.27% (48.14%-71.54%)
Negative predictive value (95% CI)	77.78% (57.73%-91.32%)
Likelihood ratio of a positive test (95% CI)	1.52 (1.17-1.96)
Post-test probability (odds) of a positive test (95% CI)	60 % (54%-66%)
Likelihood ratio of a negative test (95% CI)	0.29 (0.13-0.65)
post-test probability (odds) a negative test (95% CI)	22 % (12 %-39 %)

CI: confidence interval.

of the correlation between the expression of the three Sprouty isoforms and the presence of ascites in our cohort. Overall, 65% of patients had confirmed ascites. Our data analysis revealed an inverse correlation between the expression of Spry2 and overall ascites (*p* value: 0.028, correlation coefficient = -0.220). However, the correlation of ascites with Spry1 or Spry4 was not statistically significant (**Table 2**). Next, we categorized the patients with ascites into two subgroups for further analysis. The first subgroup consisted of 54 patients with ascites at the time of diagnosis designated as "ascites at diagnosis". In the second group named "post-treatment ascites", there were 42 cases who developed ascites after the completion of treatment. While in the former there existed no statistically significant association, a significant inverse correlation with Spry2 expression (*p* value: 0.001, correlation coefficient = -0.316) was revealed in the latter. Since the post-treatment ascites subgroup included patients with and without ascites at the time of diagnosis, we further divided this subgroup into "post-treatment only" and "pre- and post-treatment" categories. Among 46 patients with no ascites at the time of diagnosis, 11 patients developed post-treatment ascites (post-treatment only). Out of a total of 65 patients with ascites, 31 cases had ascites both at the time of diagnosis and following the treatment (pre- and post-treatment). Our data revealed a significant inverse correlation between Spry2 expression and the "post-treatment only" ascites (*p* value: 0.007, correlation coefficient = -0.390). Next, the association of Sprouty expression with ascites was evaluated in 23 patients who had ascites at the time of diagnosis but did not develop ascites after treatment, named "pre-treatment only". In these patients, no significant correlation was observed between Spry2 expression and the "pre-treatment only" ascites. As shown in **Table 2**, no significant association between the expression of Spry1 or Spry4 and ascites in the studied subgroups were found.

Expression of Spry2, but not Spry1 and Spry4, has a predictive value for the development of post-treatment ascites in EOC patients

Using the binary model for the expression of Spry1, Spry2 and Spry4, the predictive value of these isoforms in relation to the development of post-treatment ascites was assessed by univariate and multivariate logistic regression analyses of our cohort (**Table 3**). While the expression status of Spry1 (HR = 0.49; 95% CI, 0.21-1.14; *p* value: 0.101) and Spry4 (HR = 0.49; 95% CI, 0.22-1.10; *p* value: 0.085) showed no significant value for predicting post-treatment ascites, univariate analysis revealed the predictive significance of Spry2 for the development of post-treatment ascites (HR= 0.23; 95% CI, 0.08-0.64; *p* value: 0.005). Stage (*p* value: 0.020), ascites at diagnosis (*p* value: 0.001) and refractory disease (*p* value: 0.001) were also found as the significant predictors among other clinicopathological parameters.

In multivariate logistic regression analysis, Spry2 (HR = 0.25; 95% CI, 0.07-0.83; *p* value: 0.024), ascites at diagnosis (HR = 0.19; 95% CI, 0.06-0.56; *p* value: 0.003) and refractory disease (HR = 0.09; 95% CI, 0.02-0.38; *p* value: 0.001) retained their predictive value and were thus identified as the independent predictors of post-treatment ascites in EOC patients. Next, we performed receiver operating characteristic

(ROC) curve analysis to evaluate the sensitivity and specificity of the Spry2 expression status in the prediction of post-treatment ascites in our patients. The sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratios of positive and negative tests are all summarized in **Table 4**. In the "post-treatment only" group, refractory disease was found as the only predictor of ascites formation (HR = 0.10; 95% CI, 0.02-0.52; *p* value: 0.006) in univariate analysis.

There is no correlation between refractory disease and Sprouty expression

In our cohort, 21% of patients were diagnosed with refractory disease. Mean expression scores of Spry1, Spry2 and Spry4 in these patients were 2.48±0.44, 2.71±0.25 and 5.05±0.62, respectively, as compared to 3.15±0.22, 2.78±0.16 and 5.47±0.26 in non-refractory group. Despite the lower expression of the three Sprouty isoforms in this group, no statistically significant differences were found (*p* values of 0.176, 0.846 and 0.483 for Spry1, Spry2 and Spry4, respectively). As demonstrated in **Table 1**, no significant correlation was observed between the expression of Sprouty isoforms and refractoriness.

Expressions of Spry isoforms cannot predict response to chemotherapy in EOC patients

Eventually, we explored the predictive value of the expression status of Spry1, Spry2 and Spry4 for response to chemotherapy with carboplatin and taxol of our patients (**Table 3**). Spry1 showed no predictive value for response to chemotherapy (HR = 0.58; 95% CI, 0.20-1.67; *p* value: 0.320). Similarly, Spry2 (HR = 0.48; 95% CI, 0.14-1.57; *p* value: 0.225) and Spry4 (HR = 0.76; 95% CI, 0.29-1.99; *p* value: 0.579) failed to demonstrate a significantly meaningful value for predicting the refractory disease. The parameters with a significant predictive value for response to chemotherapy included tumor subtype (HR = 0.18; 95% CI, 0.04-0.88; *p* value: 0.034) and residual disease (HR = 0.22; 95% CI, 0.06-0.76; *p* value: 0.016) in univariate analysis which retained their independent significance in multivariate analysis, too (tumor subtype: HR = 0.10; 95% CI, 0.01-0.53; *p* value: 0.007; residual tumor: HR = 0.14; 95% CI, 0.03-0.56; *p* value: 0.006).

Discussion

For the past fifteen years, an expanding body of evidence has continued to support the crucial role of Sprouty proteins in normal and cancer cell biology [7]. We have recently reported the predictive value of Spry1 and Spry2 for overall survival and disease free survival of EOC patients [10, 12]. Pursuant to our previous works, the possible association between post-treatment ascites and the expression of the Sprouty protein isoforms was explored in the present study. In addition to exhibiting a negative correlation with the development of post-treatment ascites, Spry2 was identified as a marker with predictive value for the condition. To the best of our knowledge, this is the first report showing a link between Sprouty and malignant ascites. Among the three isoforms studied, only Spry2 showed an association with and a predictive value for ascites formation. In agreement, current evidence shows that Sprouty isoforms exert divergent biological effects despite their functional cooperation and structural interactions [7]. Moreover, the role of different Sprouty isoforms is associated with further complexity and even controversy in cancer. With respect to the development of post-treatment ascites in EOC, it can be postulated that Spry2 might exert an inhibitory effect by hindering the tumor growth and development, and/or through regulation of mechanisms that promote ascites formation.

Among factors with significant implication in ascites formation in EOC are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and interleukin 6 (IL-6). These three factors are known to be largely involved in the pathogenesis of EOC and all have shown mitogenic as well as pro-angiogenic activities in this disease. MAPK/ERK signaling cascades activated by VEGF and FGF are among pathways regulated by Sprouty proteins through a negative feedback loop [7]. Playing an important role in the physiology of normal ovaries, VEGF has a major contribution to the growth and development of EOC mainly through the induction of tumor angiogenesis and enhancement of vascular permeability [13]. Preclinical studies have shown that overexpression of VEGF can transform normally functional ovarian epithelium into neoplastic, ascites-producing tissue [14, 15]. Through similar mecha-

nisms, VEGF also contributes to the development of the characteristic features of the advanced EOC-associated peritoneal carcinomatosis and malignant ascites. As with VEGF, implication of FGF in the pathophysiology of EOC is well documented. Similarly, FGF has shown both angiogenic and mitogenic activities in EOC. In this regard, FGF has been reported to stimulate proliferation, migration and invasion of EOC cells *in vitro* and to promote angiogenesis *in vivo* [16-19]. These effects are believed to result, at least in part, from the regulation of other genes and proteins that contribute to the invasive and angiogenic features of malignant tumors, including urokinase-type plasminogen activator (uPA) [20], matrix metalloproteinases (MMPs) [21], VEGF [22, 23] and E-cadherin [21, 24-26]. Known as a pleotropic cytokine, IL-6 is implicated in EOC carcinogenesis. It influences EOC growth and development through direct and indirect effects on tumor cells or their microenvironment, including immune system components, respectively [27, 28]. As such, IL-6 has been indicated to promote EOC cell proliferation, migration, invasion, survival and resistance to chemotherapeutic agents [29-31]. IL-6 also contributes to EOC-induced angiogenesis [32] and malignant ascites [33].

Here, Spry2 was found to negatively correlate with post-treatment development of malignant ascites and identified as an independent predictor of the condition. Given the implications of VEGF and FGF in both MAPK/ERK signaling and malignant ascites formation, it is not unlikely that Sprouty proteins play a role in the regulation of this pathological process. However, our previous study failed to indicate a meaningful correlation between the expression levels of Spry2 and those of VEGF, FGF-2 and IL-6 in EOC [12]. Sprouty has also shown to regulate angiogenesis and vascular permeability independently of Ras/MAPK/ERK cascade. In this regard, Spry4 was implicated in Ras-independent regulation of VEGF-induced angiogenesis and vascular permeability [34]. Recently, Spry4 has also been implicated in c-Src-dependent, Ras-independent regulation of angiogenesis and vascular permeability through inhibition of endothelial cell migration and adhesion and accelerated degradation of VE-cadherin [35]. These findings and pertinent hypotheses need to be addressed in future studies and the molecular mechanisms responsible are yet to be elucidated.

In our cohort, we did not observe any correlation between Sprouty expression and refractoriness. However, since our investigation so far has revealed that low Spry2 expression is associated with poor outcome and increased risk of recurrence, death [12] and ascites development, the usefulness of Spry2 in the stratification of EOC patients for the treatment with carbotaxol can be argued. In agreement with this notion are the results from a study by Faratian et al that suggest the use of Spry2 in stratifying patients for trastuzumab therapy in breast cancer [36]. The investigators studied the expression of Spry2 in a cohort of 122 patients treated with trastuzumab and showed the decreased expression of Spry2 in association with poor outcome. In another study by Frolov et al, Spry4 was proposed as a reliable marker of the imatinib-responsive treatment in patients with gastrointestinal stromal tumors (GIST) [37]. They identified *Spry4* as an imatinib-responsive gene significantly downregulated in the treated cells and the Spry4 protein as a downstream effector of the c-Kit-activated ERK targeted by the drug. In their clinical study, since Spry4 levels were dramatically decreased in patients responsive to the drug compared with non-responsive patients, the authors proposed Spry4 as a reliable marker of the imatinib-responsive treatment. In a recent study by Li et al, however, the downregultation of Spry2 and Spry4 proteins was found to play a role in the imatinib-induced feedback activation of FGF signaling in GIST cells as an adaptation mechanism to target inhibition [38].

In conclusion, we report for the first time that the expression of Spry2 protein predicts the development of ascites in EOC following the adjuvant treatment with carbotaxol. Our findings suggest that the Spry2 expression status could be utilized for follow-up and monitoring purposes in EOC and hence for better management of the disease. Results from the present study also lay the basis for the evaluation of Spry2 protein in therapeutic approaches, including patient stratification in personalized therapy, that warrant further investigation.

Disclosure of conflict of interest

None.

Address correspondence to: Samar Masoumi-Moghaddam, Department of Surgery, Cancer

Research Laboratories, Level 3, Research and Education Center, 4-10 South Street, Kogarah, NSW 2217, Australia. Tel: 0061(2) 9113 1534; Fax: 0061(2) 9113 3967; E-mail: samar.masoumi@gmail.com

References

[1] http://globocan.iarc.fr/Pages/fact_sheets_population.aspx.

[2] Gilbert L, Basso O, Sampalis J, Karp I, Martins C, Feng J, Piedimonte S, Quintal L, Ramanakumar AV, Takefman J, Grigorie MS, Artho G and Krishnamurthy S. Assessment of symptomatic women for early diagnosis of ovarian cancer: results from the prospective DOvE pilot project. Lancet Oncol 2012; 13: 285-91.

[3] Puiffe ML, Le Page C, Filali-Mouhim A, Zietarska M, Ouellet V, Tonin PN, Chevrette M, Provencher DM and Mes-Masson AM. Characterization of ovarian cancer ascites on cell invasion, proliferation, spheroid formation, and gene expression in an in vitro model of epithelial ovarian cancer. Neoplasia 2007; 9: 820-829.

[4] Aletti GD, Gallenberg MM, Cliby WA, Jatoi A and Hartmann LC. Current Management Strategies for Ovarian Cancer. Mayo Clinic proceedings. Mayo Clinic 2007; 82: 751-770.

[5] Taylor SE and Kirwan JM. Ovarian cancer: current management and future directions. Obstetrics, Gynaecol Reprod Med 2012; 22: 33-37.

[6] Stewart DJ. Mechanisms of resistance to cisplatin and carboplatin. Crit Rev Oncol Hematol 2007; 63: 12-31.

[7] Masoumi-Moghaddam S, Amini A and Morris D. The developing story of Sprouty and cancer. Cancer Metastasis Rev 2014; 33: 695-720.

[8] Chen VW, Ruiz B, Killeen JL, Coté TR, Wu XC, Correa CN and Howe HL. Pathology and classification of ovarian tumors. Cancer 2003; 97: 2631-2642.

[9] Odicino F, Pecorelli S, Zigliani L and Creasman WT. History of the FIGO cancer staging system. Int J Gynaecol Obstet 2008; 101: 205-210.

[10] Masoumi-Moghaddam S, Amini A, Wei AQ, Robertson G and Morris DL. Sprouty 1 predicts prognosis in human epithelial ovarian cancer. Am J Cancer Res 2015; 5: 1531-1541.

[11] Kwabi-Addo B, Wang J, Erdem H, Vaid A, Castro P, Ayala G and Ittmann M. The expression of Sprouty1, an inhibitor of fibroblast growth factor signal transduction, is decreased in human prostate cancer. Cancer Res 2004; 64: 4728-4735.

[12] Masoumi-Moghaddam S, Amini A, Wei AQ, Robertson G and Morris DL. Sprouty 2 protein, but not Sprouty 4, is an independent prognostic biomarker for human epithelial ovarian cancer. Int J Cancer 2015; 137: 560-570.

[13] Masoumi Moghaddam S, Amini A, Morris DL and Pourgholami MH. Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer. Cancer Metastasis Rev 2012; 31: 143-162.

[14] Ramakrishnan S, Subramanian IV, Yokoyama Y and Geller M. Angiogenesis in normal and neoplastic ovaries. Angiogenesis 2005; 8: 169-182.

[15] Schumacher JJ, Dings RP, Cosin J, Subramanian IV, Auersperg N and Ramakrishnan S. Modulation of angiogenic phenotype alters tumorigenicity in rat ovarian epithelial cells. Cancer Res 2007; 67: 3683-3690.

[16] Crickard K, Gross JL, Crickard U, Yoonessi M, Lele S, Herblin WF and Eidsvoog K. Basic fibroblast growth factor and receptor expression in human ovarian cancer. Gynecol Oncol 1994; 55: 277-284.

[17] Lin W, Peng Z, Wang G, Bi J, Liu S and Wang H. [An experimental research in the inhibiting effect of bFGF-MAb on the growth of ovarian cancer cells and transplanted tumor]. Sichuan Da Xue Xue Bao Yi Xue Ban 2003; 34: 625-627, 693.

[18] Lin W, Peng ZL, Zheng A, Bi JH and Huang ZY. [The effect of basic fibroblast growth factor in ovarian cancer growth and angiogenesis]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2003; 20: 532-535.

[19] Zhang Y, Shang H, Sun LG, Liu N and Yu HY. [Expression of aFGF and bFGF in ovarian cancer and their effect on ovarian cancer cell proliferation]. Ai Zheng 2003; 22: 1162-1165.

[20] Li T and Jiang S. Effect of bFGF on invasion of ovarian cancer cells through the regulation of Ets-1 and urokinase-type plasminogen activator. Pharm Biol 2010; 48: 161-165.

[21] Strutz F, Zeisberg M, Ziyadeh FN, Yang CQ, Kalluri R, Muller GA and Neilson EG. Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. Kidney Int 2002; 61: 1714-1728.

[22] Giavazzi R, Sennino B, Coltrini D, Garofalo A, Dossi R, Ronca R, Tosatti MP and Presta M. Distinct role of fibroblast growth factor-2 and vascular endothelial growth factor on tumor growth and angiogenesis. Am J Pathol 2003; 162: 1913-1926.

[23] Sako A, Kitayama J, Yamaguchi H, Kaisaki S, Suzuki H, Fukatsu K, Fujii S and Nagawa H. Vascular endothelial growth factor synthesis by human omental mesothelial cells is augmented by fibroblast growth factor-2: possible role of mesothelial cell on the development of peritoneal metastasis. J Surg Res 2003; 115: 113-120.

[24] Billottet C, Elkhatib N, Thiery JP and Jouanneau J. Targets of fibroblast growth factor 1 (FGF-1) and FGF-2 signaling involved in the invasive and tumorigenic behavior of carcinoma cells. Mol Biol Cell 2004; 15: 4725-4734.

[25] Lau MT, So WK and Leung PC. Fibroblast growth factor 2 induces E-cadherin down-regulation via PI3K/Akt/mTOR and MAPK/ERK signaling in ovarian cancer cells. PLoS One 2013; 8: e59083.

[26] Wu JC, Yan HC, Chen WT, Chen WH, Wang CJ, Chi YC and Kao WY. JNK signaling pathway is required for bFGF-mediated surface cadherin downregulation on HUVEC. Exp Cell Res 2008; 314: 421-429.

[27] Heikkila K, Ebrahim S and Lawlor DA. Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. Eur J Cancer 2008; 44: 937-945.

[28] Nash MA, Ferrandina G, Gordinier M, Loercher A and Freedman RS. The role of cytokines in both the normal and malignant ovary. Endocr Relat Cancer 1999; 6: 93-107.

[29] Obata NH, Tamakoshi K, Shibata K, Kikkawa F and Tomoda Y. Effects of interleukin-6 on in vitro cell attachment, migration and invasion of human ovarian carcinoma. Anticancer Res 1997; 17: 337-342.

[30] Wang Y, Li L, Guo X, Jin X, Sun W, Zhang X and Xu RC. Interleukin-6 signaling regulates anchorage-independent growth, proliferation, adhesion and invasion in human ovarian cancer cells. Cytokine 2012; 59: 228-236.

[31] Wang Y, Niu XL, Qu Y, Wu J, Zhu YQ, Sun WJ and Li LZ. Autocrine production of interleukin-6 confers cisplatin and paclitaxel resistance in ovarian cancer cells. Cancer Lett 2010; 295: 110-123.

[32] Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, Thompson R, Schioppa T, Nemeth J, Vermeulen J, Singh N, Avril N, Cummings J, Rexhepaj E, Jirstrom K, Gallagher WM, Brennan DJ, McNeish IA and Balkwill FR. Interleukin-6 as a therapeutic target in human ovarian cancer. Clin Cancer Res 2011; 17: 6083-6096.

[33] Lo CW, Chen MW, Hsiao M, Wang S, Chen CA, Hsiao SM, Chang JS, Lai TC, Rose-John S, Kuo ML and Wei LH. IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer. Cancer Res 2011; 71: 424-434.

[34] Taniguchi K, Ishizaki T, Ayada T, Sugiyama Y, Wakabayashi Y, Sekiya T, Nakagawa R and Yoshimura A. Sprouty4 deficiency potentiates Ras-independent angiogenic signals and tumor growth. Cancer Sci 2009; 100: 1648-1654.

[35] Gong Y, Yang X, He Q, Gower L, Prudovsky I, Vary CP, Brooks PC and Friesel RE. Sprouty4 regulates endothelial cell migration via modulating integrin beta3 stability through c-Src. Angiogenesis 2013; 16: 861-875.

[36] Faratian D, Sims AH, Mullen P, Kay C, Um I, Langdon SP and Harrison DJ. Sprouty 2 is an independent prognostic factor in breast cancer and may be useful in stratifying patients for trastuzumab therapy. PLoS One 2011; 6: e23772.

[37] Frolov A, Chahwan S, Ochs M, Arnoletti JP, Pan ZZ, Favorova O, Fletcher J, von Mehren M, Eisenberg B and Godwin AK. Response markers and the molecular mechanisms of action of Gleevec in gastrointestinal stromal tumors. Mol Cancer Ther 2003; 2: 699-709.

[38] Li F, Huynh H, Li X, Ruddy DA, Wang Y, Ong R, Chow P, Qiu S, Tam A, Rakiec DP, Schlegel R, Monahan JE and Huang A. FGFR-Mediated Reactivation of MAPK Signaling Attenuates Antitumor Effects of Imatinib in Gastrointestinal Stromal Tumors. Cancer Discov 2015; 5: 438-51.