BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2017
VOL. 74, NO. 3, 133–137
https://doi.org/10.1080/09674845.2017.1278889

The clinical value of Vav3 in peripheral blood for predicting lymphatic metastasis of gastric cancer

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ABSTRACT

Background: Overexpression of *Vav3*, a gene involved in signal transduction, promotes invasion and inhibits apoptosis in several cancers. The clinical value of the protein product of this gene, Vav3, in the peripheral blood of gastric cancer patients is unknown. We hypothesised increased serum Vav3 that related to tissue levels and lymph node metastases. In addition, we further explored its clinical value in respect of linked molecules Rac-1, MMP-7 and ICAM-1

Methods: 120 gastric cancer patients who had radical surgery were enrolled. Immunohistochemistry was used to determine the expressions of Vav3, Rac-1, MMP-7 and ICAM-1 in gastric cancer mucosa and normal mucosa. ELISA was used to detect these proteins in peripheral blood of gastric cancer patients and 100 age- and sex-matched healthy controls.

Results: Vav3, Rac-1 and MMP-7 ($P < 0.001$), but not ICAM-1 ($P = 0.303$) were more highly expressed by cancer tissues than normal gastric mucosa. Serum levels of all molecules were higher than those in healthy subjects ($P < 0.001$). Levels of Vav3, Rac-1 and MMP-7 decreased 2 weeks postoperatively (all $P < 0.001$) but there was no change in ICAM-1 ($P = 0.192$). Similarly, increased levels of Vav3, Rac-1 and MMP-7 were present in patients with lymphatic metastasis than those without (all $P < 0.001$) but there was no difference in ICAM-1 levels ($P = 0.378$). There were positive correlations between Vav3 with Rac-1 and MMP-7 in cancer tissues ($P < 0.001$), and also between Vav3 and Rac-1 in pre-surgery blood ($P = 0.003$).

Conclusions: Vav3 in peripheral blood may serve as a biomarker for gastric cancer, and to predict the lymphatic metastasis in gastric cancer.

ARTICLE HISTORY
Received 2 December 2016
Accepted 2 January 2017

KEYWORDS
Gastric cancer; Vav3 gene; lymphatic metastasis; tumour biomarker

Introduction

Gastric cancer is one of the most common gastrointestinal tumours, contributing to more than 200,000 deaths in China alone each year[1, 2]. Populations are under threat due to the all-age vulnerability, early non-specific symptoms, high incidence of postoperative recurrence, metastases to lymph nodes and a low chance for complete tumour removal. In order to achieve early diagnosis and recurrence prediction efficiently and accurately, new biomarkers are needed. Recent studies have confirmed overexpression of *Vav3*, a gene involved in signal transduction, invasion and metastasis of tumour cells, in a variety of malignant tumours, such as bladder cancer, lymphoma and breast cancer.[3–8] Our previous studies have also revealed high expression of *Vav3* in gastric cancer cells, and suggested that *Vav3* might participate in the apoptosis, invasion and drug resistance[9–11].

Vav3 encodes a ~25 kDa guanine nucleotide exchange factor that acts on Rho family GTPases, such as Rac-1, RhoA and RhoG. In particular, Rac-1 is involved in the migration and invasion of cancer cells, and is regulated directly by *Vav3*[12]. Matrix metalloproteinase-7 (MMP-7, also known as Matrilysin-1, a 28 kDa proenzyme) is linked to the promotion of the invasion of cancer cells via FasL and of apoptosis via TNF-α,[13] and *Vav3* may suppress expression of MMP-7.[9] Intercellular adhesion molecule-1 (ICAM-1) at the cell surface influences metastasis and adhesion of tumour cells,[14] and it could be regulated by Rac-1 in endothelial cells.[15]

A link between the expression of *Vav3* in cancer tissues and levels of its protein product in the peripheral blood is unclear and may have clinical value. In view of the multiple functions of *Vav3* in the progression of gastric cancer, and high levels of Vav3 protein detected in gastric cancer tissues, we hypothesised that there might be increased Vav3 protein in peripheral blood of gastric cancer patients. If so, serum Vav3 protein could be a new indicator of tumour load in patients. To further characterise the clinical and oncological science of Vav3, we also determined the expression of Rac-1, MMP-7 and ICAM-1 in the tumours, normal tissues and in peripheral blood and in patients with lymph node metastases.

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Materials and methods

One hundred and twenty patients diagnosed as gastric cancer and having undergone radical surgery at the Fourth Hospital of Hebei Medical University between January 2015 and December 2015 were enrolled, including 82 males and 38 females, with mean (standard deviation) age of 53.8 ± 13.7 years. Postoperative pathology reports were reviewed to confirm the preoperative diagnosis. One hundred healthy volunteers as controls matched in age and gender were recruited over the same period. Fasting blood specimens were collected from patients one day before and two weeks after surgery, and only once from controls. Paraffin specimens of tumour tissues from the patients were sampled, with another 50 specimens of normal gastric mucosa collected from Department of Pathology as controls. All participants had signed the informed consents. This study was approved by the Behavioural and Social Sciences Ethical Review Committee, the Fourth Hospital of Hebei Medical University.

Detection of expressions of Vav3, Rac-1, MMP-7 and ICAM-1: Serial sections of paraffin specimens (4 μm) were detected by immunohistochemistry (IHC) and DAB reagent kit (Sigma-Aldrich Inc., Shanghai, China) to determine the expressions of Vav3, Rac-1, MMP-7 and ICAM-1 proteins in the gastric cancer tissues of patients and normal tissues of controls. The primary antibodies of Vav3, Rac-1, MMP-7 and ICAM-1 (Santa Cruz, CA, USA) were prepared and diluted as 1:100 (Vav3) and 1:200 (Rac-1, MMP-7 and ICAM-1). A confirmed pathological section was applied as positive control, with phosphate buffered saline as negative control, respectively. Positive staining of Vav3 and Rac-1 protein was located in cytoplasm and cytomembrane. MMP-7, ICAM-1 protein was positively stained mainly in cytoplasm, partially in cytomembrane. Strength of positivity was determined by the percentile of positive cells in the tumour tissue and the staining intensity. Levels of the four proteins in the blood specimens of gastric cancer patients and controls were detected using ELISA kits (R&D Systems, Minneapolis, MN, USA). Both IHC (Envision method) and ELISA assays were performed according to the manufacturers' instructions.

Statistical analysis: Differences in the expressions of Vav3 with Rac-1, MMP-7 and ICAM-1 proteins in tissues were determined with chi-square (χ^2) test. Differences in levels of serum proteins (reported as mean and standard deviation) were tested with Student *t* test. The correlations between the expression of Vav3 with Rac-1, MMP-7 and ICAM-1 were assessed using Spearman's rank correlation coefficient. All statistical analysis were performed using Statistical Product and Service Solutions, version 18 for Windows (SPSS, Inc., Chicago, IL, USA), with $P < 0.05$ regarded as significant.

Results

Tissue expression of Vav3, Rac-1, MMP-7 and ICAM-1: As summarised in Table 1 and Figure 1, the rates of Vav3, Rac-1 and MMP-7 in cancers were 71.7, 65.8 and 59.2%, respectively, all higher than that in the normal gastric tissues (14.0, 18.0 and 24.0%, respectively; all $P < 0.001$). Moreover, the positive rates of the three proteins were much higher in patients with lymphatic metastasis (81.8, 76.1 and 69.3%) than in the patients without (43.8, 37.5 and 31.3%, respectively; all $P < 0.001$). However, no significant difference was observed between ICAM-1 in gastric cancer and normal mucosa tissues ($P = 0.303$), and between with and without lymphatic metastasis in gastric cancer tissues ($P = 0.559$).

Serum Vav3, Rac-1, MMP-7 and ICAM-1: Each of these molecules was significantly higher in the pre-surgery blood specimens from patients with gastric cancer than those from the controls ($P < 0.001$) (Table 2, Figure 2a). Compared to control serum levels, Vav3, Rac-1, MMP-7 and ICAM-1 were 4.3-, 2.5-, 2.9- and 1.6-fold increased, respectively. The expressions of Vav3, Rac-1 and MMP-7 decreased two weeks postoperatively (all $P < 0.001$), at which point there was no significant difference compared with controls ($P = 0.369$, 0.923, 0.073, respectively). In contrast, the expression of ICAM-1 was still at a similar level in the post-surgery blood specimens ($P = 0.192$, compared with pre-surgery). The concentrations of Vav3 (1.4-fold), Rac-1 (1.5-fold) and MMP-7 (1.9-fold) were higher in patients with lymphatic metastasis than in those without ($P < 0.001$), while no significant difference was observed for ICAM-1 between the two groups ($P = 0.378$) (Table 2, Figure 2b).

Spearman's rank correlation analysis identified positive correlations between expressions of Vav3, Rac-1 and MMP-7 in cancers and their counterparts in peripheral blood, with a coefficient correlations (*r* value) of 0.34, 0.41 and 0.33, respectively ($P < 0.001$). This suggests higher levels of Vav3, Rac-1 and MMP-7 in peripheral blood is consistent with those in gastric cancer tissues. However, no such correlation was present regarding ICAM-1 ($P = 0.204$). In cancer tissues, positive correlations also existed between Vav3 and Rac-1, Vav3 and ICAM-1, as well as Rac-1 and MMP-7 ($r = 0.30$, 0.30 and 0.35, respectively, $P < 0.05$); while in the peripheral blood similar correlation was only present between Vav3 and Rac-1 ($r = 0.27$, $P = 0.003$).

Table 1. Expressions of Vav3, Rac-1, MMP-7, ICAM-1 in gastric cancer.

		Gastric cancer (n = 120)	Normal mucosa (n = 50)	Gastric cancer without lymphatic metastasis (n = 32)	Gastric cancer with lymphatic metastasis 2 (n = 88)
Vav3	Positive	86	7	14	72
	Negative	34	43	18	16
Rac-1	Positive	79	9	12	67
	Negative	41	41	20	21
MMP-7	Positive	71	12	10	61
	Negative	49	38	22	27
ICAM-1	Positive	51	17	15	36
	Negative	69	33	17	52

Data are the number of positive results. P value from χ^2. All $P < 0.001$ except ICAM gastric cancer v normal controls ($P = 0.303$) and ICAM with/without lymphatic metastases ($P = 0.559$).

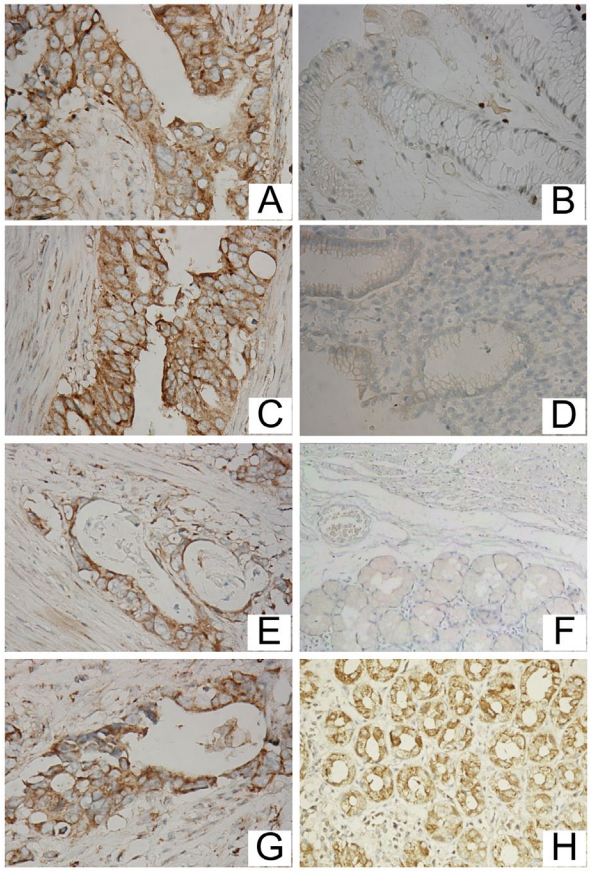

Figure 1. Vav3, Rac-1, MMP-7 and ICAM-1 in gastric cancer and normal tissues.

Expression of Vav3, Rac-1, MMP-7 and ICAM-1 proteins with immunohistochemistry (IHC×400), and positive staining locations were in cytoplasm and/or cytomembrane. Expressions of these proteins in gastric cancer and normal tissues were shown as A-H: Expressions of Vav3 protein in gastric cancer and normal tissues were shown as A and B, expressions of Rac-1 protein were shown as C and D, MMP-7 protein as E and F, ICAM-1 protein as G and H.

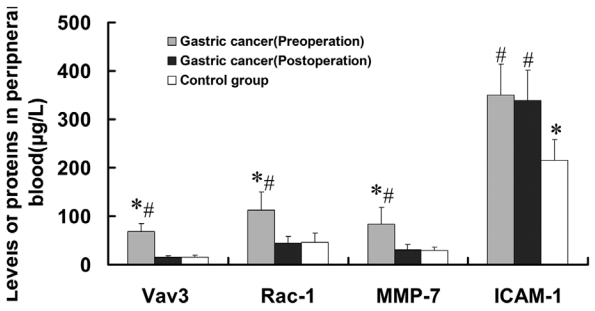

Figure 2a. Levels of Vav3, Rac-1, MMP-7 and ICAM-1 in peripheral blood of gastric cancer patients and control group.

Levels of all molecules were higher than those in control group ($P < 0.05$). *$P < 0.05$ versus postoperative group; # $P < 0.05$ versus Control group.

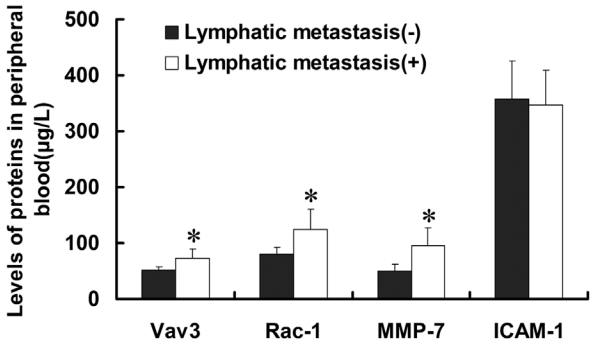

Figure 2b. Preoperative levels of Vav3, Rac-1, MMP-7 and ICAM-1 in peripheral blood of gastric cancer with different lymphatic metastasis.

Levels of Vav3, Rac-1, MMP-7 proteins were higher in lymphatic metastasis(+) group than those in lymphatic metastasis(-) group. *$P < 0.05$ versus lymphatic metastasis(-) group.

Table 2. Levels of Vav3, Rac-1, MMP-7, ICAM-1 in the peripheral.

	Pre-operative gastric cancer (n = 120)	Post-operative gastric cancer (n = 120)	Controls (n = 100)	Pre-operative gastric cancer free of lymphatic metastases (n = 32)	Pre-operative gastric cancer with lymphatic metastases (n = 88)	P
Vav3	67.7 ± 16.4[a]	15.5 ± 2.2[b]	15.9 ± 3.4[d]	52.1 ± 5.3	73.4 ± 15.4	<0.001
Rac-1	112.4 ± 37.1[a]	45.0 ± 14.0[b]	45.2 ± 19.7[e]	80.7 ± 11.5	124 ± 36.5	<0.001
MMP-7	83.5 ± 34.6[a]	31.1 ± 11.2[b]	28.7 ± 7.8[f]	50.7 ± 11.9	95.4 ± 32.3	<0.001
ICAM-1	349 ± 64[a]	339 ± 63[c]	215 ± 43 [g]	358 ± 68	346 ± 63	0.378

Data mean with standard deviation, units μg/L. [a]$p < 0.001$ compared to controls (t test). [b]$p < 0.001$. [c]$p = 0.192$ versus pre-operative (paired t test). [d]$p = 0.369$. [e]$p = 0.923$. [f]$p = 0.073$. [g]$p < 0.001$ compared to post-operative levels (t test).

Discussion

The carcinogenesis and progression of gastric cancer is complex, with the participation of multiple genes.[16] Previous studies have confirmed a variety of proteins associated with gastric cancer, and demonstrated their roles in the carcinogenesis and metastasis of cancer cells. One of these, Vav3, a GDP/GTP exchange factor, could activate the genes in terms of RhoA, Rac-l, CDc42, etc. [17–19] It has also been reported that *Vav3* is associated with cancer growth, invasion and metastasis. Although the regulation of *Vav3* in breast cancer, lymphoma and other malignant cancers have been documented;[20–24] few studies has reported a role in gastric cancer and its value as a biomarker.

We observed higher expression of Vav3 in the gastric cancer tissue than in normal gastric mucosa tissues, which was even higher in patients with lymphatic metastasis. This suggests that Vav3 may have a role in the genesis and metastasis of gastric cancer. To confirm a role for Vav3 in gastric cancer, we determined serum Vav3 levels before and after surgery, finding higher levels of Vav3 in the pre-surgery blood of patients with gastric cancer than healthy controls, which decreased significantly to the normal level after radical surgery. The relative increase in mean Vav3 (4.3-fold) was higher than the comparator molecules (1.6–2.9-fold). We further defined a role for Vav3 in that serum levels and tissue expression is higher in patients with lymphatic metastasis than those free of metastases. Moreover, the positive correlation of Vav3 concentrations between cancers and peripheral blood suggested that Vav3 could be regarded as a biomarker to reflect the development of gastric cancer.

To further evaluate the possible regulation pathways of Vav3, we studied the association between Vav3 and three other cancer-related proteins: Rac-1, product of a gene directly regulated by *Vav3*; MMP-7, a critical promoter in the invasion and metastasis of cancer cells by eroding the histological barriers around by; ICAM-1, a regulator involved in the development and metastasis of cancers by regulating the adhesion between cells or between cells and matrixes.[25–30] Spearman's rank correlation coefficient revealed positive correlation between Vav3 and Rac1 in both cancers and peripheral blood, which suggested the role of Vav3 in gastric cancer is by regulating the expression of Rac-1. While the positive correlation between Vav3 and ICAM-1 in cancer tissues and lack of a significant correlation in peripheral blood suggested that the regulation capacity of Vav3 to cancer-related proteins varied in the development of gastric cancer. However, more research is needed to further investigate the underling molecular mechanisms.

These findings indicate the importance of Vav3 in the genesis and lymphatic metastasis of gastric cancer, and also suggest that Vav3 may have clinical value as a biomarker. However, our study just revealed the correlation between Vav3 in different tissues of gastric cancer, while more experiments *in vitro* and *in vivo* are needed to confirm the underlying molecular mechanism. In conclusion, our work is an advance in biomedical science because it shows that the levels of Vav3 protein are different in peripheral blood of gastric cancer patients and healthy controls, and difference was also detected in peripheral blood of gastric cancer patients with and without lymphatic metastasis.

Summary

What is known about this subject
- There are no reliable serum markers regarding the high incidence of postoperative recurrence in gastric cancer.
- *Vav3* is involved in signal transduction, invasion and metastasis of many cancers.
- *Vav3* gene has been confirmed with increased expression in gastric cancer tissues and cell lines.

What this paper adds
- Higher level of serum Vav3 in the pre-surgery blood in patients with gastric cancer than in healthy controls.
- Higher level of Vav3 in pre-surgery blood of patients with lymphatic metastasis than without.
- Effect of Vav3 in gastric cancer might be related to its interaction with Rac-1 and ICAM-1.

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