RESEARCH ARTICLE

Aberrant Expression of Pim-3 Promotes Proliferation and Migration of Ovarian Cancer Cells

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Abstract

Pim kinase-3(Pim-3), a member of serine/threonine protein kinases, has been implicated in multiple human cancers and involved in Myc-induced tumorigenesis. However, little is known regarding its expression and biological function in human ovarian cancer. In this study we showed that the clinical significance and biological functions of Pim-3 in ovarian cancer and found that higher Pim-3 mRNA level are detected in ovarian cancer tissues than those in normal ovarian tissues. There are significant correlations between higher Pim-3 expression levels with the FIGO stage, histopathological subtypes, and distant metastasis in ovarian cancer patients. Lentivirus-mediated gene overexpression of Pim-3 significantly promotes the proliferation and migration of SKOV3 cell lines. Furthermore, MACC1 and Pim-3 expression were significantly correlated in human ovarian cancer cells, and overexpression of Pim-3 in ovary cancer cells increased MACC1 mRNA and protein expression. The data indicate that Pim-3 acts as a putative oncogene in ovary cancer and could be a viable diagnostic and therapeutic target for ovarian cancer.

Keywords: Pim-3 - MACC1 - expression - tumorigenesis - ovarian cancer

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Introduction

Ovarian cancer is highly fatal gynecological cancer. The morbidity of ovarian cancer, following endometrial cancer, has surpassed cervical cancer, ranked at the second place in female genital system cancer. It is the fifth leading related death of cancer in women with 14, 030 deaths each year in the United States. The 5-year relative survival rate is about 43% (Siegel et al., 2013). Early stage patients can receive radical cancer resection, which provides good prognoses. Ovarian cancer at its early stages (I/II) is difficult to be diagnosed until it spreads and advances to later stages (III/IV). This is because most symptoms are non-specific and thus of little use in diagnosis.With further invasion and distant metastasis of ovarian cancer, the prognosis in advanced stage patients is still unsatisfactory. Therefore, it is of great importance to identify genes and regulatory mechanisms conferring the malignant potential to ovarian cancer cells that could enhance the understanding of cancer progression and result in the development of new therapeutics.

Tumor metastasis is a complicated multistage process in which tumor cells leave the original site, migrate into other tissues or organs through the lymphatic system or bloodstream, and then form secondary tumors which are similar to the original tumors. In the late stages of cancer, tumor metastasis is very common and responsible for the majority of cancer deaths. Provirus integrating site Moloney murine leukemia virus (Pim) family belongs to the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) group and exhibits serine/threonine kinase activity (Mukaida et al., 2011). Pim-3 was initially identified as a depolarization-induced gene in a rat pheochromocytoma cell line PC12 and was designated as kinase induced by depolarization (KID)-1 (Feldman et al., 1998). KID-1 was renamed as Pim-3 because it showed high sequence similarity with Pim-1 and Pim-2, members of the proto-oncogene Pim family (Konietzko et al., 1999). Previous studies showed that Pim-3 was aberrantly expressed in a wide variety of tumors (Mukaida et al., 2011). Similar to Pim-1 and Pim-2, Pim-3 can prevent cell apoptosis and promote cell survival and protein translation, thereby enhancing cell proliferation of normal and malignant cells. An analysis of tumor tissues specifically from gastric cancer patients showed that Pim-3 expression was significantly associated with gastric cancer invasion and metastasis (Zheng et al., 2008). And Pim-3 was also associated with sarcoma-induced bone invasion (Narlik-Grassow et al., 2012). The inhibition of Pim-3 by shRNA also reduced endothelial cell spreading, vascular tube

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formation, and migration of prostate cancer (Nakano et al., 2012). Pim-3 can be regulated by the Ets family of transcription factors in NIH3T3 cells and human Ewing's sarcoma cells (Deneen et al., 2003). Later studies showed that Pim-3 is activated by the Ets-1 transcription factor in pancreatic cancer cells (Li et al., 2009). And Pim3 is a Myc target gene. Inhibition of Pim kinases induces cell death of Myc-induced lymphomas (Forshell et al., 2011). These data support the idea that Pim-3 can contribute to the metastasis and invasion of tumors (Zhang et al., 2009). However, whether Pim-3 is aberrantly expressed in ovarian cancer and the relationship between Pim-3 and ovarian cancer has not been reported yet.

Metastasis-associated in colon cancer-1 (MACC1) has been reported to promote tumor proliferation and invasion mediated via hepatocyte growth factor (HGF)/ mesenchymal-epithelial transition factor (c-Met) signaling in colorectal cancer (Stein et al., 2009; Galimi et al., 2011; Migliore et al., 2012). Recently, a clinical study showed that aberrant overexpression of MACC1 may indicate poor prognosis of ovarian cancer patients for early recurrence and distance metastasis (Zhang et al., 2014). Down-regulation of MACC1 in OVCAR-3 cells resulted in significant inhibition of cell proliferation, migration and invasion, meanwhile obvious enhancement of apoptosis which might be caused by the induced inhibition of HGF/ Met and MEK/ERK pathways (Zhang et al., 2011).

The similar roles of Pim-3 and MACC1 in cancer metastasis aroused our curiosity to investigate whether there are relationship between Pim-3 and MACC1 during the procedure of cancer metastasis. In our study, we aimed to examine the role of Pim-3 in ovarian cancer and the potential mechanisms involved by a retrospective analysis of 26 patients' ovarian cancer specimens and clinicopathological parameters as well as by carrying out cell experiments to clarify the influence of Pim-3 on ovarian cancer proliferation and invasion and its effect on the MACC1.

Materials and Methods

Cell culture

The human ovarian cancer cell lines HO8910, SKOV3 and OVCAR3 were obtained from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in a PRMI1640 with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin/streptomycin (Invitrogen) at 37°C in an atmosphere containing 5% CO_2.

Patients

A total of 26 patients diagnosed with International Federation of Gynecology and Obstetrics (FIGO) stage I to IV ovarian cancer tissues and 16 normal ovarian tissues were studied from 2012 through 2013 at General Affiliated Hospital to Tianjin Medical University (Tianjin, China). These ovarian cancer patients were the subjects of various histopathological parameter reviews. All slides were reclassified and graded by 1 pathologist according to World Health Organization criteria; there were 13 serous cystadenocarcinoma, 7 Mucinous cystadenocarcinoma, 3 clear cell tumour and 3 Dysgerminoma. The normal ovarian tissues were obtained from oophorectomy of the patients of benign uterine diseases, such as uterine fibroid. Informed consent to use the samples for diagnostic and research purposes were obtained according to the procedures established at our institution. Clinicopathological variables including age, histological grade, FIGO stage, ascites and distant metastasis were abstracted from the medical records of each patient.

RNA preparation and analysis

Total RNA was isolated from ovarian cancer cells and tissues using Trizol (Invitrogen, USA) according to manufacturer's instructions. Reverse transcription reactions were performed with 1μg total RNA using FastQuant RT kit (TIANGEN, China). The expression of Pim-3 was quantified by SYBR qPCR Kit (TIANGEN, China). Expression data were uniformly normalized to β-actin as an internal control, and the relative expression levels were evaluated using the ΔΔCt method. The primer sequences used for qRT-PCR are as follows: *i)* Pim-3 (F): 5`-CTCATCGACTTCGGTTCGG-3`; *ii)* Pim-3 (R): 5`-TATCGTAGAGAAGCACGCCC-3`; *iii)* MACC1 (F): 5`- GGCATGCTCATGCAAACACA-3`; *iv)* MACC1 (R): 5`-TAAACACTGGCAGGCAACCA-3`; *v)* β-actin (F): 5`-CATGTACGTTGCTATCCAGGC-3`; *vi)* β-actin (R): 5`-CTCCTTAATGTCACGCACGAT-3`.

Protein extraction and western blotting

Cell lysates were prepared by incubation for 30 minutes on ice with lysis buffer (50 mM Hepes, pH 7.5, 120mM NaCl, 1 mM EDTA, 2.5mM EGTA, 0.1% Tween-20, 1mM PMSF, 1mM NaF, 1mM Na_3VO_4, 10mM β-glycerophosphate supplemented with Minicomplete protease inhibitor cocktail tablets (Roche)), followed by sonication at 3×7sec pulses in a Soniprep 150 MSE, 30% power. Cell debris was centrifuged at 4°C, 12, 000g for 15 minutes. Protein concentration of the supernatants was measured using Pierce BCA Protein Assay Kit (Thermo Scientific, USA). 50 μg of proteins were separated by electrophoresis in SDS-PAGE and transferred to a PVDF membrane (Millipore). After blocking with 5% nonfat milk (DB, France) at room temperature for 1 hour, the membrane was incubated with indicated antibody at 4°C overnight. The membranes were then washed with TBS-Tween (10mM Tris-HCl, pH 7.6, 150mM NaCl and 0.05% Tween-20) containing 5% milk. The membranes were developed by enhanced chemiluminescence using the Super Signal West Dura or Pico reagents (Pierce) and an X-ray film to detect the protein of interest.

Generation of expression vector and stable transfection

DNA fragments encoding the complete coding sequence of human Pim-3 were amplified by PCR from creating flanking XbaI and NotI restriction sites and were cloned in the expression vector pCDH lentivector (System Biosciences). The primer sequences used for Pim-3 cloning are as follows: Forwards: 5'-CTAGTCTAGACGGCCACCATGCTGCTCTCCAA GTTCGG-3' Reverse: 5'-ATAAGAATGCGGCCGCGG CAAGCTCTCGCTGCTGGAC-3'.

SKOV3 cells were approximately 60% confluence to be transfected with Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Stable clones were selected with puromycin (0.5μg/ul) starting at 48 hours after transfection. All transfected cell lines were then assayed for overexpression of Pim-3 via qRT-PCR and western blotting.

Cell viability

The impact of overexpression of Pim-3 on the ovarian cancer cell proliferation was measured by Cell Counting Kit-8 (CCK-8), according to the manufacturer's instruction. Briefly, ovarian cancer cells were cultured in 96-well plates. 10μl of CCK-8 reagent was added to each well at different time points. The absorbance was measured at 450 nm after 3 hours of incubation at 37°C. All experiments were done with four wells per experiment and repeated at least three times.

Wound healing assay

Cells were seeded in 6-well plates (7.5×10^5cells/well) and allowed to adhere for 24h. The cells were kept serum-free PRMI1640 overnight for starvation. Then the cells were washed with phosphate buffer saline (PBS), scratched with a pipette tip in the middle of the plate, and then washed with PBS to remove the cells which had detached during the scratch. After washing with PBS, media was added containing 10% FBS. Wound closure was monitored microscopically at different time points and photographed at 0 and 24 hours.

Statistical analysis

The difference between each sample and vector control was assessed by either Multi-way ANOVA test or Student t test. The X^2 test or Fisher exact probability test were used to compare clinicopathological features of the ovarian cancer tissues and normal ovarian tissues with *Pim-3* mRNA levels and *MACC1* mRNA expression levels. Correlation between *Pim-3* and *MACC1* mRNA expression levels was evaluated using Spearman correlation analysis. Statistical analysis was performed with SPSS statistical software (SPSS Statistics 20). All statistical tests were two-sided and P values were considered statistically significant for $p<0.05$.

Results

Differential expression of Pim-3 mRNA and protein in normal and ovarian cancer tissues and ovarian cancer cell lines

To determine the potential role of Pim-3 in ovarian cancer progression, we evaluated Pim-3 expression in ovarian cancer and normal ovarian tissue by qRT-PCR (Figure 1a). Remarkably, the expression level of Pim-3 is significantly higher in cancer tissue versus normal tissue (2.88±4.57 *vs* 0.028±0.015, p=0.010).

Also we conducted qRT-PCR analysis in three ovarian cancer cell lines HO8910, SKOV3 and OVCAR3 cell lines. We found that *Pim-3* mRNA expression level was highest in OVCAR3 cells than that of HO8910 and SKOV3 cells, and results were confirmed by western blotting (Figure 1b, c).

Relationships between Pim-3 expression level and clinicopathological parameters in ovarian cancers

The relationships between Pim-3 expression levels and clinicopathological parameters are shown in Table 1. The Pim-3 overexpression was found to be significantly correlated with the FIGO stage (p=0.024), histopathological subtypes (p=0.031), and distant metastasis (p=0.032), but not with age (p=0.529), ascites (p=0.065), which indicating a potential role of Pim-3 overexpression in promoting aggressive phenotypes in ovarian cancer in some extent.

Overexpression of Pim-3 promotes the proliferation and migration in ovarian cancer cells

Over-regulation of Pim-3 expressions: Our clinicopathological findings indicate that Pim-3 expression is associated with ovarian cancer metastasis, then we hypothesized that Pim-3 is involved in the regulation

Table 1. Association between Pim-3 Expression Level and Clinicopathological Parameters

Parameter	Category	No. of cases	Pim-3 mRNA	P value
Age (years)	≤55	12	2.25±3.34	0.529
	>55	14	3.42±5.48	
FIGO stage	I/II	17	0.87±0.48	0.024*
	III/IV	9	6.68±6.3	
Histopathological-subtypes	G1	7	0.84±0.58	0.031*
	G2/G3	19	3.63±5.16	
Ascites	Absent	12	1.32±1.74	0.065
	Present	14	4.54±5.77	
Distant Metastasis	Absent	9	0.86±0.62	0.032*
	Present	17	3.95±5.37	

*Statistically significant

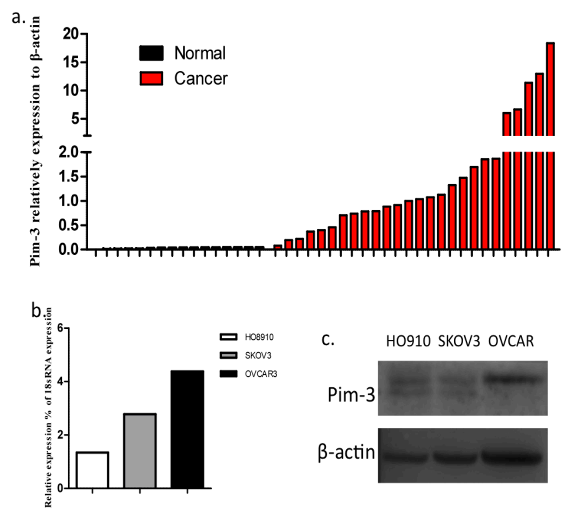

Figure 1. Pim-3 Expression Levels are Elevated in Human Ovarian Cancer Tissues and High Metastatic Potential Cell Lines. a) qRT-PCR analysis of Pim-3 expression level in 42 cases of ovary tissue(n=26 in ovarian cancer group, n=16 in normal ovarian tissue group). Pim-3 kinase levels were normalized to that of β-actin. **b)** A statistical plot of the average Pim-3 expression level of ovary cancer and normal ovarian tissue. **c)** Pim-3 mRNA expression level in HO8910, SKOV3 and OVCAR3 cells. d. Western blotting showed Pim-3 protein expression level in HO8910, SKOV3 and OVCAR3 cells

of ovarian cancer cells metastasis. To unravel the role of Pim-3 in ovarian cancer cells metastasis, we over-expressed Pim-3 in SKOV3 cells. After transfection 48 h, transfected cells with green fluorescence under fluorescence microscopy were observed (Figure 2a). Expressions of Pim-3 in stably transfected cells, which were selected by puromycin, were measured by western blotting. Compared to vector control cells, levels of Pim-3 protein were significantly up-regulated in SKOV3-Pim-3/pCDH cells (Figure 2b).

Pim-3 promotes SKOV3 cells proliferation

To further evaluate whether Pim-3 gene up-regulation in SKOV3 cells promotes cell proliferation, cell viability was determined by CCK-8 assay at 24h, 48h, and 72h after plating. As shown in Figure 3a, upregulation of Pim-3 expression significantly improved the cell viability of SKOV3 cells in a time-dependent manner (p<0.001, Figure 3a). After 1 day of subculure, the viability of pCDH-Pim-3 group didn't show obvious difference between Pim-3 group and vector control group (p=0.081). After cultured for 48h and 72h, the percentage of viable cells in pCDH-Pim-3 group markedly increased, as compared with the negative controls (p=0.000 and p=0.000, respectively). Thus, over-expression of Pim-3 significantly increased the proliferation of SKOV3 cells compared with that of control then.

Over-expression of Pim-3 enhances SKOV3 cells migration

Wound healing assay was conducted to study Pim-3 contribution to *in vitro* cell migration. We found that the migratory potential of Pim-3 upregulated SKOV3 cells was significantly increased compared with those of the control SKOV3 cells transfected with the empty plasmid (Figure 3b) (p=0.035).

Pim-3 affects MACC1 protein expression

Previous studies have shown that MACC1 had important roles in tumor cell migration and invasion, *MACC1* mRNA expression might be an independent prognostic indicator of recurrence in colorectal carcinoma (Stein et al., 2009). We assessed whether Pim-3 upregulation in human ovarian cancer tissues and metastatic potential ovarian cancer cell lines had an influence on the expression of *MACC1* gene. Western blotting results showed that the mRNA expression levels of *MACC1* were increased in Pim-3 overexpression SKOV3 cells compared to the vector control cells (Figure 4a). In order to investigate whether there is correlative effect between *Pim-3* expression level and *MACC1* expression level in ovarian cancer tissues, qRT-PCR analysis was also conducted to examine mRNA expression levels of *MACC1* in the same samples of ovarian cancer tissues and normal ovarian tissues. The expression level of *MACC1* is significantly higher in cancer tissue versus normal tissue [58.0772 (inter-quartile range: 3.2970-306.5656) *vs* 1.3571 (inter-quartile range: 0.8897-55.9535)] (p=0.002) (Figure 4b). Results also showed that *MACC1* mRNA expression levels was positively correlated with those of Pim-3 expression levels, with a linear regression line and Spearman correlation significance (R^2=0.784, p<0.01) (Figure 4c).

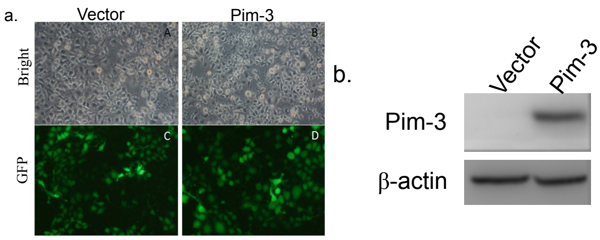

Figure 2. Transfection of Pim-3 into Ovarian SKOV3 Cells. a. SKOV3 cells under incandescent light and fluorescent light (×100) after transfection 48h. b. Western blotting showed higher expression level of Pim-3 in pCDH-Pim-3 group compared to empty vector group

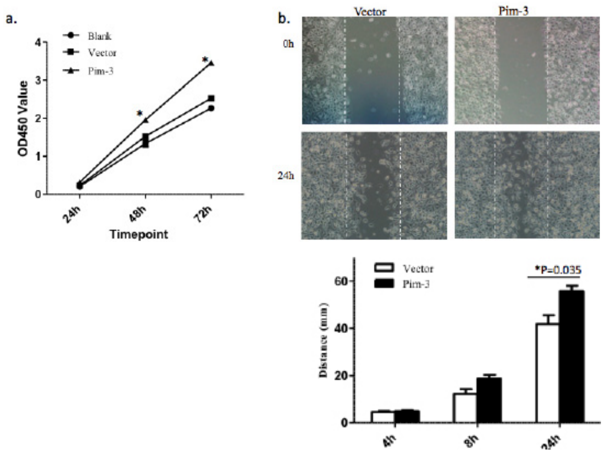

Figure 3. Pim-3 Exhibiting Proto-oncogenic Property in SKOV3 Cell Line. a. Cellular proliferation of untransfected or transfected SKOV3 cells. Cell viability was increased in pCDH-Pim-3 group after 24h of transfection, as compared with nontransfected SKOV3 cells group and pCDH vector group (P <0.05). **b.** Migration of cells was assessed by the wound healing assay at 0, 4, 8, and 24h. The data of wound healing assay were then averaged and summarized as width ratio of migratory inhibition (p=0.035)

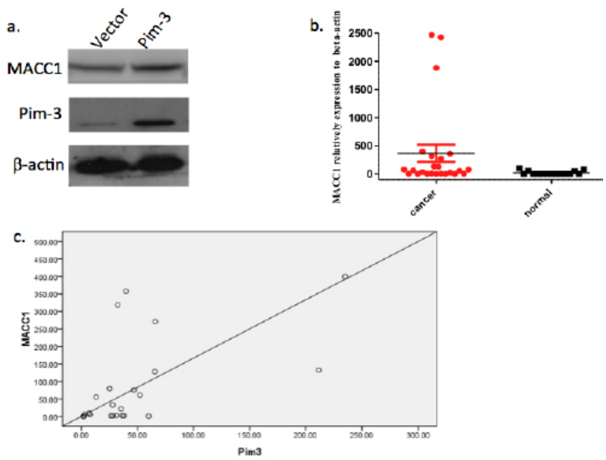

Figure 4. Pim-3 Upregulated MACC1 Expression a. MACC1 expression was upregulated in Pim-3 overexpression SKOV3 cells detected by western blot. **b.** Box plots illustrated median and inter-quartile range of MACC1 mRNA expression levels in 42 cases of ovary tissue(n=26 in ovarian cancer group, n=16 in normal ovarian tissue group. c.The mRNA level of *MACC1* mRNA was significantly correlated with *Pim-3* mRNA expression in ovarian cancer samples

Discussion

Our results document three important new findings. Firstly, that analyzing the expression of Pim-3 in 26 ovarian cancer and 16 normal ovarian tissue specimens as well as cancer patients' relative clinicopathological parameters, we show that Pim-3 is overexpressed in ovarian cancer tissue compared to that in the normal tissue. Pim-3 overexpression is associated with a lower histopathological subtype and cancer metastasis. Secondly, up-regulation of Pim-3 in SKOV3 cells increases the proliferation, and *in vitro* migratory potential of ovarian cancer cells. Thirdly, Pim-3 expression levels have influence to that of MACC1.

Compared to advanced ovarian cancer, the early stage ovarian cancer patients might have better prognosis, even cured, after an aggressive conventional treatments. Therefore, it is important to find novel methods that can effectively identify ovarian cancer at early stage and inhibit cancer cell growth and metastasis. Pim-3, a proto-oncogene with serine/threonine kinase activity, has been confirmed aberrantly expressed in various malignant solid tumors, but not normal tissues of endoderm-derived organs such as the colon, stomach, liver and pancreas (Fujii et al., 2005; Li et al., 2006; Popivanova et al., 2007; Zheng et al., 2008). However, Pim-3 has never been linked to ovarian cancer yet. In this study, we confirmed for the first time that significantly increased Pim-3 expression levels was detected in the vast majority of ovarian cancer tissues when compared with those in normal ovary tissues (Figure 1). Moreover, in cancer patients, upregulation of Pim-3 expression level is significantly correlated with the FIGO stage (p<0.05), histopathological subtypes (p<0.05) and metastasis (p<0.05). Recently, several studies reported that Pim-3 could promote cancer invasion and metastasis in gastric and pancreatic cancer, sarcoma and other tumors (Nakano et al., 2012; Zhang et al., 2013; Lou et al., 2014). These findings regarding the role of Pim-3 in different cancers support the clinical results of our study, implying that Pim-3 may ubiquitously promote cancer invasion and metastasis.

To determine whether metastatic characteristics initiated by Pim-3, we analyzed the expression of Pim-3 in 3 kinds of ovarian cancer cell lines (Figure 1b, c). Our study showed that all three ovarian cancer cell lines expressed high levels of Pim-3, implicating that Pim-3 may play a causative role in metastatic characteristic intensifying.

To further evaluate the biological significance of Pim-3 in ovarian cancer invasion and metastasis, we established Pim-3 overexpression cell lines to effectively and specifically increase endogenous Pim-3 expression in SKOV3 cell line. We observed that, in SKOV3 cells, Pim-3 overexpression markedly promoted cell proliferation and migration respectively (Figure 3). These findings are consistent with a report by Yang et al., in which down-regulation of Pim-3 gene inhibited the migration and proliferation of endothelial cells (Yang et al., 2011). Recently, Wang et al. (2014) proposed that Pim-3 could affect the proliferation, differentiation and apoptosis of liver cancer cells and facilitate the occurrence and development of cancers by inducing the STAT3 signaling pathway and regulating the expression of apoptosis related genes and VEGF-overexpression. These characteristics might contribute to Pim-3 associated aggressive biological behaviors of ovarian cancer. Further studies are required to elucidate the mechanism by which Pim-3 mediates invasion and metastasis in ovarian cancer cells.

MACC1 was first identified as a colon cancer oncogene that promotes proliferation and metastasis. It associates with peritoneal metastasis and an advanced stage of TNM classification in colorectal carcinomas (Stein et al., 2009; Stein et al., 2012). Recent studies have also linked MACC1 upregulation to cancer development and metastasis in lung adenocarcinoma, hepatocellular carcinoma, breast cancer, gastric cancer and gallbladder cancer (Shimokawa et al., 2011; Stein et al., 2012). Researchers have shown that MACC1 mediates various biological functions, including angiogenesis, cell growth, cell differentiation, as well as cellular motility and invasion, by regulating the hepatocyte growth factor (HGF)/MET signaling pathway (Stein et al., 2012). In ovarian cancer, overexpressed MACC1 was detected in the vast majority of ovarian cancer tissues when compared with normal and benign ovarian tissues. And the aberrant expression of MACC1 was correlated with lymph nodes metastasis, shorter overall survival time, higher FIGO stage, and histological grade. Moreover, down-regulation of MACC1 resulted in obvious inhibition of cell proliferation and metastasis. Meanwhile down-regulation of MACC1 significant enhanced apoptosis of OVCAR-3 cells (Zhang et al., 2011). And our studies also showed *MACC1* mRNA expression levels are upregulatedin ovarian cancer tissues compared to those of normal ovarian tissues (Figure 4b), which is consistent with the studies by Zhang et al (Zhang et al., 2011). On the basis of these findings, it is implicated that MACC1 is a promising therapeutic target for anti-metastatic and anti-tumor intervention strategies of solid cancers (Stein, 2013). Basing on the similar functions of MACC1 and Pim-3 in regulating cancer metastasis, it is reasonable to speculate that modulation of ovarian cancer invasion and metastasis by Pim-3 would have effect on the expression level of MACC1.Indeed, in this study, we found that significant up-regulation of MACC1 expression was detected in Pim-3 overexpression SKOV3 cells (Figure 4). Moreover, we found that the expression levels of Pim-3 in ovarian cancer tissues was positively correlated with those of MACC1 (Figure 4, R^2=0.784, p<0.01), implicating that Pim-3 and MACC1 may play a synergy role in ovarian cancer invasion and metastasis. Our findings provided a potential mechanism for MACC1 dysregulation and contribution to ovarian cell invasion. It may help to estimate the therapeutic utility of Pim-3 in ovarian cancer cells. However, their precise roles and molecular mechanisms remain to be further studied in the future.

Since Pim kinase expression has been associated with poor outcome in several different tumor types (Wright et al., 2003; Dave et al., 2006; Rossi et al., 2006; Zheng et al., 2008; Peltola et al., 2009; Warnecke-Eberz et al., 2009) and chemoresistance have been seen in tumor cells overexpressing the Pim kinases (Zemskova et al., 2008; Behan et al., 2009; Chen et al., 2009; Mumenthaler et al., 2009), inhibition of Pim kinases has evoked a lot of interest and inspired efforts has been conducted to inhibit the Pim kinases as a potential tragedy of cancer treatment. Moreover, the triple knockout Pim kinase mice are viable and fertile, which suggests that inhibition of the Pim kinase family could be possible without severe side effects (Mikkers et al., 2004). By now, several independent groups have developed small-molecule inhibitors against Pim kinases (Akue-Gedu et al., 2012; Gavara et al., 2013). Stemonamide synthetic intermediates derivative can inhibit Pim-3 as well as Pim-1 and Pim-2 activities and can reduce tumor growth *in vivo* xenograft models using a human pancreatic cancer cell line without causing major adverse effects (Li et al., 2010; Wang et al., 2013). Since our results showed Pim-3 might be involved in ovarian cancer proliferation and metastasis, Pim-3 might also be an ideal target for ovarian cancer therapy.

In summary, the present study showed that Pim-3 might be involved in ovarian cancer proliferation and metastasis. The classification of patients according to Pim-3 expression levels provides a valuable tool with which to identify ovarian cancer patients with poor prognoses. Our findings might also extend our knowledge of the biological progression of ovarian cancer and could provide a new therapeutic target for ovarian cancer. However, it is unknown how Pim-3 regulates MACC1 expression in ovarian cancer cells. Future study will be needed to confirm the relationship between the expressions of these two genes and to elucidate the underlying mechanism.

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