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ORIGINAL ARTICLE

Clinical and therapeutic relevance of PIM1 kinase in gastric cancer

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Abstract
Background Gastric cancer is a leading cause of cancer-related mortality, and chemotherapeutic options are currently limited. PIM1 kinase, an oncogene that promotes tumorigenesis in several cancer types, might represent a novel therapeutic target in gastric cancer.
Methods We studied the expression and genomic status of PIM1 in human primary gastric normal and tumor tissue samples by immunohistochemistry and array-based comparative genomic hybridization (aCGH). To ascertain whether PIM1 expression predicted susceptibility to PIM1 kinase-specific inhibition, the cytotoxic effect of a previously reported PIM1-specific small molecular inhibitor (K00135) was investigated in two gastric cancer cell lines with high (IM95) and undetectable (NUGC-4) PIM1 expression levels.
Results PIM1 expression was exclusively nuclear in normal gastric epithelial cells, while aberrant expression/localization (decreased nuclear and/or increased cytoplasmic expression) was observed in 75.6% (68/90) of the human gastric cancer tissue samples, with a significant inverse correlation between nuclear and cytoplasmic expression levels. Clinicopathological analyses revealed that decreased nuclear PIM1 expression correlated with poorer survival and greater depth of tumor invasion, while increased cytoplasmic PIM1 expression correlated inversely with the presence of lymphovascular invasion. High-level *PIM1* amplification was identified in 10.5% of gastric cancers by aCGH. K00135 impaired the survival of IM95, while it had no significant effect on NUGC-4 survival.
Conclusion Our findings demonstrate the clinical and therapeutic relevance of PIM1 in gastric cancers, and suggest that PIM1 represents a potential therapeutic target.

Keywords PIM1 · Gastric cancer · Targeted therapy · Amplification

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Introduction

Gastric cancer is a major cause of cancer-related morbidity and mortality, being the second leading cause of cancer-related deaths globally [1]. A significant number of patients

present with advanced disease, leading to poor survival outcomes [2]. To date, the number of effective molecular therapeutics available against gastric cancer is limited [3]. There is a need to identify potential novel therapeutic targets in gastric cancer.

PIM1 is a serine/threonine kinase [4] that was initially discovered to play an oncogenic role in T-cell lymphomagenesis in mice [5]. The oncogenetic mechanisms of PIM1 include the promotion of cell cycle progression [6, 7], the suppression of apoptosis [8], and the enhancement of drug resistance [9]. Overexpression of PIM1 also results in genomic instability and polyploidy [10, 11]. PIM1 has been implicated in tumorigenesis in many cancer types, including myeloid leukemias [12], B-cell lymphomas [13, 14], and prostate [15] and head and neck cancers [16], and there has been growing interest in developing small molecular inhibitors of PIM1 as potential therapeutic agents [17–20].

PIM1 expression has been reported to correlate with prognosis in gastric cancer [21, 22]; however, its potential as a candidate therapeutic target has not been explored in detail. Of note, the *PIM1* gene is located on chromosome 6p21 [23], a region that is amplified in some gastric cancers [24]. In this study, our objectives were to study the immunohistochemical expression of PIM1 in gastric cancer tissue samples, identify whether genomic *PIM1* amplification was present in primary gastric cancer tissue samples and cell lines, and determine whether PIM1 is a candidate therapeutic target by performing drug testing in cell lines using a previously reported potent and specific small molecular inhibitor of PIM1, K00135 [12].

Materials and methods

Clinical samples

National University Hospital (NUH)

Ninety surgically resected specimens of primary human matched gastric tumor and normal samples were selected from the archives of the Department of Pathology, National University Hospital, Singapore. Tissue microarray construction, immunohistochemistry and clinicopathological analyses were performed on this cohort. Twenty-four matched biopsies with intestinal metaplasia were analyzed by immunohistochemistry on complete sections. Patient consent for research, and ethics approval from the Institutional Review Board were obtained.

National Cancer Centre of Singapore (NCCS)

One hundred and five anonymized primary human gastric tumors were obtained from the NCCS tissue repository, with approval from the local Ethics Committee and signed patient informed consent. Array-based comparative genomic hybridization (aCGH) was performed on this cohort. Tumor content in all samples was confirmed to be >50% by frozen sections. Genomic DNAs from pooled blood lymphocytes obtained from healthy female and male subjects were used as normalization controls.

Tissue microarray construction and immunohistochemistry

Tissue cylinders with a diameter of 1 mm were punched from formalin-fixed, paraffin-embedded samples of tumor and normal areas of the donor tissue blocks and deposited into a recipient block using a tissue-arraying instrument (Beecher Instruments®, Silver Spring, MD, USA). Paraffin tissue microarray sections of 4 μm were deparaffinized and hydrated in graded ethanols. Heat-induced epitope retrieval of tissues was performed by boiling at 120°C in pH 6 citrate buffer target retrieval solution (Dako, Glostrup, Denmark) for 5 min in a pressure cooker. Non-specific signal was blocked by peroxidase block (Dako) for 10 min at room temperature, followed by protein block (Dako) for 30 min at room temperature. Anti-PIM1 primary monoclonal antibody (Epitomics, Burlingame, CA, USA) was incubated at room temperature for 1 h in a humidified chamber, followed by horseradish peroxidase (HRP)-conjugated secondary antibody (Dako) incubation at room temperature for 30 min. Antibody binding was revealed by diaminobenzidine (DAB) (Dako). Tissue sections were counterstained with hematoxylin, dehydrated in graded ethanols, and mounted.

For the immunohistochemical analysis, the intensity of PIM1 expression in gastric cancers was scored relative to that in normal gastric epithelial cells. Therefore, nuclear PIM1 expression was scored as being either strong or decreased, while cytoplasmic PIM1 expression was scored as being either increased or weak/undetectable.

Clinicopathological data and analysis

Clinicopathological data comprising distant metastasis, lymphovascular invasion, perineural invasion, lymph nodal metastasis, tumor size, depth of invasion, and survival were available for 76 cases from the NUH cohort. Differences between these clinical parameters and PIM1 immunohistochemical expression were assessed using the Fisher's exact test and, whenever appropriate, the χ^2 test. Their correlations were examined using Spearman's rank coefficient test. Odds ratio was estimated by means of cross-tabulation analysis. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. A Cox proportional hazards model for the hazard ratio

was used for the evaluation of survival in a multivariate analysis. All statistical analyses were performed using an SPSS package (version 15.0 for Windows, SPSS Inc., Chicago, IL, USA) with significance set at the 5% level.

Array-CGH assays and copy number analysis

One hundred and five primary gastric tumors were profiled using Agilent Human Genome 244A CGH Microarrays (Agilent Technologies, Santa Clara, CA, USA) containing approximately 240,000 distinct 60-mer oligonucleotide probes spanning the entire human genome. Sample labeling and hybridizations were performed according to the manufacturer's instructions. Hybridized slides were scanned on an Agilent DNA Microarray Scanner (Agilent Technologies) and images were extracted using Agilent Feature Extraction software. Copy number variation levels for the *PIM1* locus were analyzed using Nexus software (http://www.biodiscovery.com/index/nexus).

Cell culture

Two gastric cancer cell lines, IM95 and NUGC-4, were maintained in advanced Dulbecco's modified Eagle medium (DMEM) (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 2% Glutamax (Invitrogen) and heat-inactivated fetal bovine serum (Invitrogen), and Advanced RPMI 1640 (Invitrogen) supplemented with 1% Glutamax and 2% heat-inactivated fetal bovine serum, respectively. A prostate cancer cell line, PC-3 (ATCC, Manassas, VA, USA), was cultured in F-12 nutrient mixture (HAM) (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum under a humidified atmosphere of 5% CO_2 at 37°C.

Quantitative real-time polymerase chain reaction (PCR)

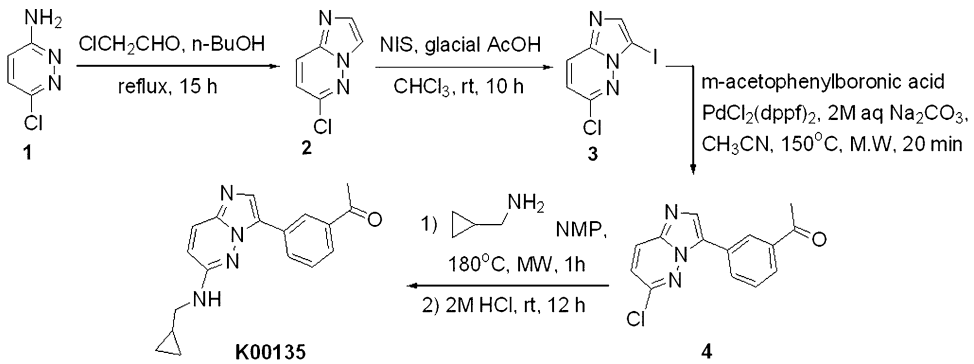
Total RNAs of IM95, NUGC-4, and PC-3 were isolated with an RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions and reverse transcribed using High Capacity cDNA Reverse Transcription (RT) kit with RNase inhibitor. Quantitative real-time PCR (Q-PCR) was performed with an ABI Prism 7900HT thermocycler (Applied Biosystems, Foster City, CA, USA), using *PIM1* and *GAPDH* FAM dye-labeled TaqMan® probes based on TaqMan® fluorescence methodology. All the reactions were conducted in duplicate. All reagents used for RT and Q-PCR were purchased from Applied Biosystems. *PIM1* mRNA was normalized to *GAPDH* mRNA.

Western blotting

Cells were lysed with protease inhibitor in NP-40 lysis buffer, briefly sonicated on ice, and subjected to high-speed centrifugation for 1 min. Cell lysates were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membranes were blocked in 5% skimmed milk in phosphate-buffered saline 0.1% Tween-20 (PBS-T) and proteins were probed with anti-PIM1 (Epitomics) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) primary antibodies, followed by HRP-conjugated anti-rabbit or anti-mouse secondary antibody. The probed proteins were detected with Luminol (Santa Cruz) on exposure to X-ray film.

Fluorescence in situ hybridization (FISH)

Cells were harvested and fixed with Carnoy's fixative (3:1 methanol:acetic acid) on coated slides. The cells were digested with protease solution (4 mg/ml protease in 0.2 N HCl) (Vysis, Abbott Molecular, Des Plaines, IL, USA) for 15 min at 37°C. A *PIM1* probe (BAC clone RP11-200O9; Enzo Life Sciences, Farmingdale, NY, USA) and a *CEP6* probe (Vysis, Abbott Molecular) were added. Denaturation and hybridization were performed using ThermoBrite (Abbott Molecular, Des Plaines, IL, USA). Following overnight hybridization at 37°C, the slides were washed in 50% formamide/2× saline-sodium citrate (SSC) at 37°C for 20 min and 1× SSC at room temperature for 15 min



Scheme 1 Synthesis of K00135

and counterstained with 125 ng/ml 4,6-diamidino-2-phenylindole (DAPI) (Vysis, Abbott Molecular). The slides were analyzed using an Olympus BX61 microscope (Olympus, Tokyo, Japan) equipped with DAPI, Spectrum Orange, Spectrum Green, and double-band pass filters for simultaneous visualization of the Spectrum Green and Spectrum Orange signals. Images were captured using CytoVision version 3.93 software (Applied Imaging, Newcastle upon Tyne, UK). One hundred cells were scored and the ratio of *PIM1* to *CEP6* signals was calculated. Gene amplification was defined by a mean *PIM1*/*CEP6* copy number of >2.2 or by a mean *PIM1* copy number of >6 per cell according to previously reported criteria [25].

K00135 synthesis

Briefly, 1-(3-(6-(cyclopropylmethylamino)imidazo[1,2-b]pyridazin-3-yl)phenyl)ethanone (K00135) was synthesized as described in Scheme 1. Treatment of 3-amino-6-chloropyridazine **1** with chloroacetaldehyde gave intermediate 6-chloroimidazo[1,2-b]pyridazine **2**, which was subsequently selectively iodinated at the 2-position with *N*-iodosuccinimide (NIS) to yield the key intermediate **3**. Suzuki coupling of **3** with *m*-acetophenylboronic acid under microwave irradiation occurred selectively at the 2-iodo position to provide intermediate **4**. Reaction of **4** with cyclopropylmethylamine under microwave conditions followed by treatment with dilute HCl afforded the target compound K00135. A detailed description of the synthetic process can be found in the Supplementary Information.

Treatment with K00135

Cells in 90 µl medium were seeded (3,000 cells/well) onto 96-well microtiter plates (Nunc, Rochester, NY, USA). After 24 h, 10 µl of medium containing K00135 with graded concentrations ranging from 10 to 10,000 nM was added to the wells. The effect on cell numbers was assessed using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) (MTS) CellTiter 96® (Promega, Madison, WI, USA) assay at 72 h post-treatment. The IC_{50} was calculated as the drug

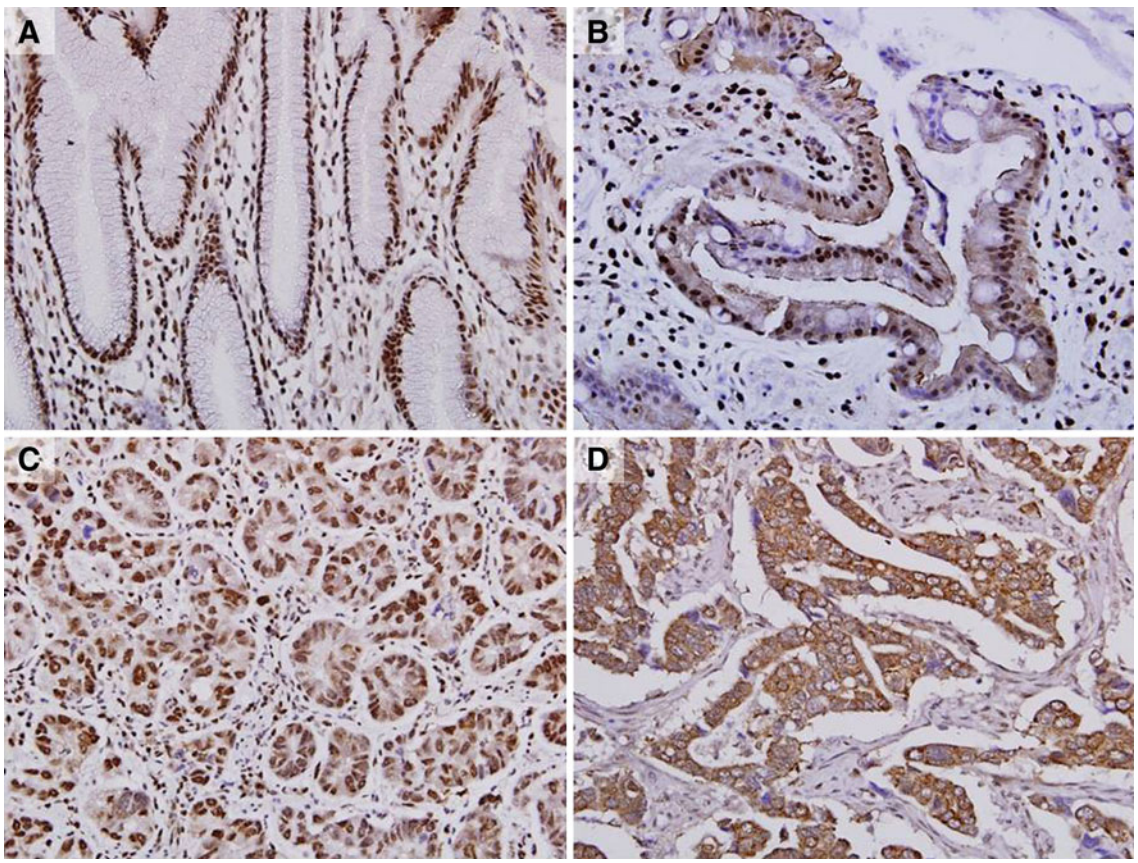


Fig. 1 PIM1 immunohistochemistry in normal and tumor gastric samples showing **a** strong nuclear staining in normal gastric epithelial cells, **b** nuclear and cytoplasmic staining in intestinal metaplasia, **c** strong nuclear staining in tumor cells, **d** strong cytoplasmic staining in tumor cells (original magnification x400)

concentration that inhibited cell proliferation by 50% compared with vehicle controls.

Results

Aberrant PIM1 expression/localization in primary gastric cancers and clinicopathological correlation

In normal gastric epithelial cells, PIM1 expression was strongly nuclear with undetectable or very weak cytoplasmic staining (Fig. 1a). Nuclear PIM1 expression was present in all cases of intestinal metaplasia, with 45.8% (11/24) showing concomitant cytoplasmic staining (Fig. 1b). In primary gastric cancer samples from the NUH cohort, we observed aberrant PIM1 expression/localization, manifested as either decreased nuclear expression or increased cytoplasmic expression (Fig. 1c, d), in 75.6% (68/90) of gastric cancers (Table 1). There was a significant inverse correlation between nuclear and cytoplasmic PIM1 expression levels ($p = 0.033$).

Complete clinicopathological information was available for 76 cases from the NUH cohort. Table 2 illustrates the demographic and clinicopathological characteristics of the NUH cohort. When analyzed against clinicopathological parameters, decreased nuclear PIM1 expression correlated with poorer survival ($p = 0.036$) (Fig. 2a) and greater depth of tumor invasion ($p = 0.003$). Multivariate analysis demonstrated a significant correlation between decreased nuclear PIM1 expression and poorer survival ($p = 0.025$). Increased cytoplasmic PIM1 expression correlated inversely with the presence of lymphovascular invasion ($p = 0.047$). No correlation between cytoplasmic PIM1 expression and survival was identified (Fig. 2b). Tables 3, 4, and 5 provide detailed analyses of nuclear and cytoplasmic PIM1 expression against the various clinicopathological parameters.

Table 1 PIM1 nuclear and cytoplasmic expression in gastric cancer samples

	Strong nuclear expression	Decreased nuclear expression	Total
Increased cytoplasmic expression	9	31	40
Weak/undetectable cytoplasmic expression	22	28	50
Total	31	59	90

Focal high-level PIM1 genomic amplification in primary gastric cancers

As gains of chromosome 6p21 have been reported in approximately 10% of gastric cancers previously [24], we were interested in investigating whether *PIM1* genomic amplification was present in our primary tumors. By aCGH analysis, *PIM1* was found to be amplified in 10.5% (11/105) and 24.8% (26/105) of the primary gastric cancers, using a threshold of high and low stringency, respectively (Fig. 3).

K00135 cytotoxicity correlates with PIM1 mRNA and protein expression levels in gastric cancer cell lines

The endogenous levels of PIM1 mRNA and protein were measured by Q-PCR and immunoblot assays. The IM95 and NUGC-4 gastric cancer cell lines displayed high and low/undetectable endogenous PIM1 expression, respectively, both at the mRNA (Fig. 4a) and protein levels (Fig. 4b). As

Table 2 Demographic and clinicopathological characteristics of National University Hospital (NUH) cohort

Parameters	*n* (%)
Age (years)	
<65	30 (39.5)
≥65	46 (60.5)
Gender	
Male	56 (73.4)
Female	20 (26.3)
Stage	
I and II	38 (50.0)
III and IV	38 (50.0)
Distant metastasis	
Absent	68 (89.5)
Present	8 (10.5)
Lymphovascular invasion	
Absent	32 (42.1)
Present	44 (33.9)
Perineural invasion	
Absent	50 (65.8)
Present	26 (34.2)
Lymph nodal metastasis	
Absent	22 (28.9)
Present	54 (71.1)
Tumor size	
<5 cm	36 (47.4)
≥5 cm	40 (52.6)
Depth of invasion (pT)	
1 and 2	44 (57.9)
3 and 4	32 (42.1)

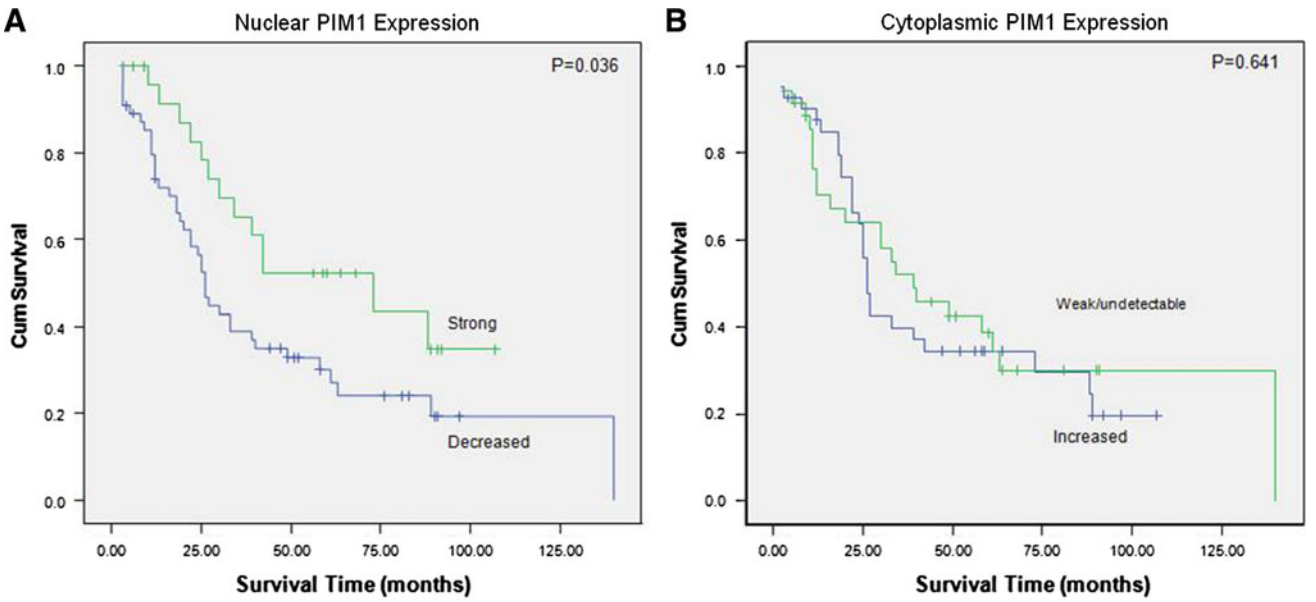


Fig. 2 Kaplan–Meier survival curve showing the relationship of **a** nuclear and **b** cytoplasmic PIM1 expression with survival. *Cum* cumulative

Table 3 Clinicopathological parameters in relation to nuclear PIM1 expression

Parameters	Decreased nuclear PIM1 expression	Strong nuclear PIM1 expression	*p* value
Stage			
I and II	25	13	0.805
III and IV	27	11	
Distant metastasis			
Absent	47	21	0.702
Present	5	3	
Lymphovascular invasion			
Absent	27	17	0.141
Present	25	7	
Perineural invasion			
Absent	36	14	0.437
Present	16	10	
Lymph nodal metastasis			
Absent	16	6	0.787
Present	36	18	
Tumor size			
<5 cm	23	13	0.466
≥5 cm	29	11	
Depth of invasion (pT)			
1 and 2	24	20	**0.003**
3 and 4	28	4	

Value in bold is significant

Table 4 Clinicopathological parameters in relation to cytoplasmic PIM1 expression

Parameters	Weak/undetectable cytoplasmic PIM1 expression	Increased cytoplasmic PIM1 expression	*p* value
Stage			
I and II	19	19	0.490
III and IV	22	16	
Distant metastasis			
Absent	36	32	0.719
Present	5	3	
Lymphovascular invasion			
Absent	13	19	**0.047**
Present	28	16	
Perineural invasion			
Absent	28	22	0.619
Present	13	13	
Lymph nodal metastasis			
Absent	12	10	0.947
Present	29	25	
Tumor size			
<5 cm	19	17	0.846
≥5 cm	22	18	
Depth of invasion (pT)			
1 and 2	26	18	0.291
3 and 4	15	17	

Value in bold is significant

PIM1 gene amplification might have accounted for the differential expression, FISH analysis was performed to ascertain this. By FISH analysis, most cells in both cell lines showed a similar number of *PIM1* and *CEP6* signals (2–4 signals) per cell (Fig. 4c), with colocalization of both *PIM1* and *CEP6* signals. Occasionally in both cell lines, the *PIM1* signals did not colocalize with the *CEP6* signals, suggesting

translocation of the *PIM1* gene to regions outside chromosome 6. No significant difference in the *PIM1* gene copy number was discerned between IM95 (*PIM1/CEP6* ratio 1.09) and NUGC-4 (*PIM1/CEP6* ratio 1.1). The mean *PIM1* copy numbers/cell were 2.2 and 2.5 for IM95 and NUGC-4, respectively, excluding the presence of *PIM1* amplification in either cell line.

K00135 has previously been reported to be a potent and specific inhibitor of PIM1, with an IC_{50} of 0.12 μmol/l [12]. To investigate whether PIM1 affected cell growth, we exposed the gastric cancer cell lines to various concentrations of the drug and measured cell viability subsequently. K00135 impaired the survival of IM95 (IC_{50} = 2.4 μmol/l) (Fig. 4d), while it had no effect on NUGC-4 gastric cancer cells (IC_{50} >10 μmol/l).

Table 5 Multivariate analysis for survival according to clinicopathological features and nuclear PIM1 expression

Parameter	HR	95% CI	*p*
Age ≥65 years	1.76	0.916–3.365	0.090
Distant metastasis	3.765	1.414–10.03	**0.008**
Lymphovascular invasion	0.801	0.386–1.661	0.551
Perineural invasion	0.562	0.266–1.187	0.131
Lymph nodal metastasis	0.520	0.227–1.190	0.121
Tumor size ≥5 cm	1.45	0.710–2.962	0.308
Depth of invasion (pT3/4)	1.418	0.730–2.753	0.302
Decreased nuclear PIM1 expression	2.45	1.127–5.348	**0.025**

HR hazard ratio, *CI* confidence interval

Values in bold are significant

Discussion

Two studies have earlier pointed to a link between PIM1 and gastric carcinogenesis [21, 22], and our present study builds on their findings by providing a more detailed immunohistochemical, clinicopathological, genomic analysis with in vitro functional studies. In contrast to Warnecke-Eberz et al. [21], who reported undetectable to weak cytoplasmic PIM1 expression in normal gastric epithelial cells, we detected strong nuclear PIM1 expression in normal gastric epithelial cells. We feel that technical factors might account for the observed difference—a polyclonal antibody was employed in their study, while a monoclonal antibody (the specificity of which was confirmed on Western immunoblotting) was utilized in ours.

We observed aberrant expression/localization of PIM1 in a population of primary gastric cancer samples, manifested as either decreased nuclear or increased cytoplasmic expression. Decreased nuclear expression correlated with greater depth of tumor invasion and poorer survival, while

Fig. 3 Copy number profiles of *PIM1* in 33 primary gastric tumors. Overlap of diverse amplification regions across multiple samples covers *PIM1*. Eleven tumors with high-level copy number amplification of *PIM1* are shown in *red*. The *color gradient* depicts the extent of copy number amplification. Genomic position and surrounding genes are indicated accordingly

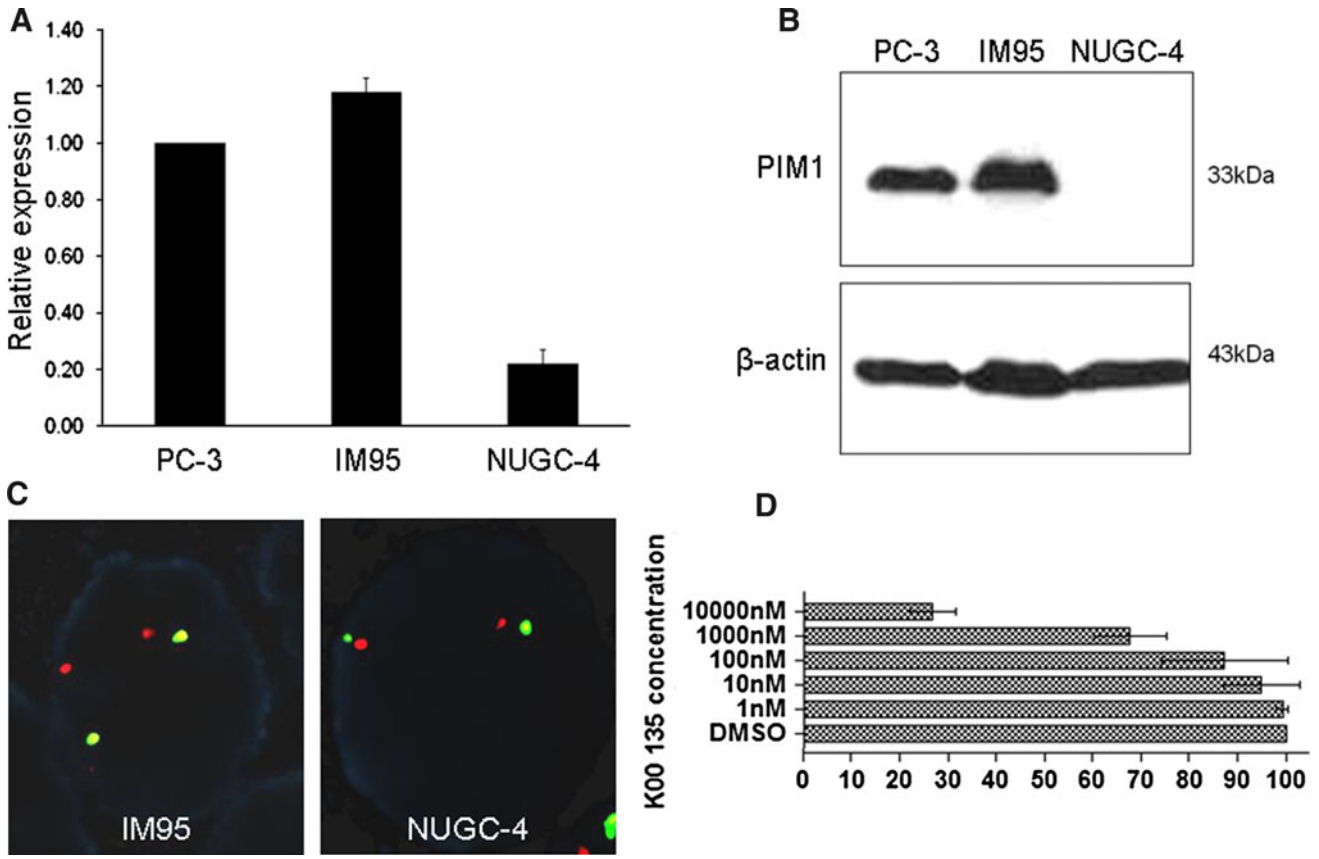


Fig. 4 Endogenous PIM1 **a** mRNA and **b** protein expression levels in IM95 and NUGC-4 cell lines. PC-3 was included as a positive control for PIM1 expression for both quantitative real-time polymerase chain reaction (Q-PCR) and Western blotting (WB). **c** *PIM1* fluorescence in situ hybridization (FISH) in IM95 and NUGC-4. *Orange* and *green signals* represent *PIM1* and *CEP6* probes, respectively. **d** Effect of K00135 treatment on IM95

increased cytoplasmic expression correlated inversely with the presence of lymphovascular invasion. In particular, multivariate analysis showed decreased nuclear PIM1 expression to be a strong independent prognostic factor. Increased cytoplasmic PIM1 expression was identified in some cases of intestinal metaplasia, a pre-malignant condition [26], suggesting that aberrant PIM1 expression occurs early during gastric carcinogenesis. Our findings raise the question as to the biological function of subcellular PIM1 localization, and its role in gastric tumorigenesis.

PIM1 is known to phosphorylate both nuclear and cytoplasmic target proteins. Exclusively nuclear target proteins include histone H3 [27], while exclusively cytoplasmic target proteins include Bad [8, 28] and BCRP/ABCG2 [9]. Target proteins that may localize in either the nucleus or cytoplasm include Runx3 [29], $p21^{Cip1/WAF1}$ [30], C-TAK1 [6, 31], $p27^{Kip1}$ [7], and C-myc [32]. Although PIM1 has been shown to promote oncogenesis through the phosphorylation of nuclear target proteins such as histone H3, based on our observation that normal gastric epithelial cells display strong nuclear PIM1 expression, it is unlikely that nuclear PIM1 functions as an oncogene in gastric cancers. Loss of nuclear expression and gain of cytoplasmic expression appear to be the likely mechanisms of oncogenesis in gastric cancers, although further work will be needed to verify this hypothesis. Our finding that cytoplasmic PIM1 expression correlates inversely with the presence of lymphovascular invasion is potentially interesting in view of a recent report that implicates PIM1 in invasion and metastasis [33].

The identification of high-level *PIM1* genomic amplification by aCGH suggests that overexpression and subsequent gain of PIM1 function might occur in a subset of gastric cancers. It is interesting to note that *PIM1* genomic amplification has been previously reported in a subset of B-cell non-Hodgkin's lymphomas [14]. It will be important in future work to elucidate the functional significance of *PIM1* genomic amplification, as this phenomenon suggests oncogenic dependency on PIM1 and therefore raises the possibility that PIM1 might be a potential molecular therapeutic target in a subset of gastric cancers. Such a paradigm is exemplified by HER2 in breast [34] and gastric cancers [35].

As an extension of our findings from the clinical samples, we therefore also sought to understand whether PIM1 expression had therapeutic significance in gastric cancers. Specifically, we wished to study whether the expression of PIM1 mediated cell survival in gastric cancers, and whether this expression predicted response to anti-PIM1-specific therapy, the latter objective being especially important because we also noted that 31.1% (28/90) of gastric cancers showed decreased nuclear and low/undetectable cytoplasmic expression which might predict decreased sensitivity or resistance to PIM1-specific treatment. Our in vitro work with gastric cancer cell lines showed that PIM1 expression mediated survival, and PIM1 expression levels correlated with susceptibility to PIM1-specific treatment. Together, these findings suggest that PIM1 expression in gastric cancers might have therapeutic relevance. The observation that the differential PIM1 mRNA and protein expression in IM95 and NUGC-4 gastric cancer cell lines was not due to a *PIM1* gene copy number difference suggests that other mechanisms, such as epigenetic or post-translational regulation [36], might regulate PIM1 levels.

In summary, we report aberrant PIM1 expression/localization and high-level *PIM1* genomic amplification in a population of gastric cancer tissue samples, and that susceptibility to PIM1 inhibition following treatment with a PIM1-specific small molecular inhibitor correlates with PIM1 mRNA and protein expression in gastric cancer cell lines. Our findings demonstrate the clinical and therapeutic relevance of PIM1 in gastric cancers, warranting further study of PIM1 as a potential therapeutic target.

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