Salt-Inducible Kinase 1 (SIK1) Is Induced by Gastrin and Inhibits Migration of Gastric Adenocarcinoma Cells

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Abstract
Salt-inducible kinase 1 (SIK1/Snf1lk) belongs to the AMP-activated protein kinase (AMPK) family of kinases, all of which play major roles in regulating metabolism and cell growth. Recent studies have shown that reduced levels of SIK1 are associated with poor outcome in cancers, and that this involves an invasive cellular phenotype with increased metastatic potential. However, the molecular mechanism(s) regulated by SIK1 in cancer cells is not well explored. The peptide hormone gastrin regulates cellular processes involved in oncogenesis, including proliferation, apoptosis, migration and invasion. The aim of this study was to examine the role of SIK1 in gastrin responsive adenocarcinoma cell lines AR42J, AGS-Ga and MKN45. We show that gastrin, known to signal through the Gq/G11-coupled CCK2 receptor, induces SIK1 expression in adenocarcinoma cells, and that transcriptional activation of SIK1 is negatively regulated by the Inducible cAMP early repressor (ICER). We demonstrate that gastrin-mediated signalling induces phosphorylation of Liver Kinase 1B (LKB1) Ser-428 and SIK1 Thr-182. Ectopic expression of SIK1 increases gastrin-induced phosphorylation of histone deacetylase 4 (HDAC4) and enhances gastrin-induced transcription of c-fos and CRE-, SRE-, AP1- and NF-kB-driven luciferase reporter plasmids. We also show that gastrin induces phosphorylation and nuclear export of HDACs. Next we find that siRNA mediated knockdown of SIK1 increases migration of the gastric adenocarcinoma cell line AGS-Ga. Evidence provided here demonstrates that SIK1 is regulated by gastrin and influences gastrin elicited signalling in gastric adenocarcinoma cells. The results from the present study are relevant for the understanding of molecular mechanisms involved in gastric adenocarcinomas.

Introduction
Gastrin is a regulatory peptide hormone which plays a crucial role in integration of exocrine and endocrine functions in the gastrointestinal tract. We and others have shown that gastrin regulates several important cellular processes in the gastric epithelium and in adenocarcinoma cells including proliferation [1], anti-apoptosis [2,3,4], migration [5] and invasion [6]. Functional genomics approaches have identified a range of new gastrin target genes [3,4,7,8,9]. By genome wide gene expression profiling we found that gastrin induces transcription of Salt-inducible kinase 1 (SIK1/Snf1lk) in the pancreatic adenocarcinoma cell line AR42J [4]. SIK1 is a member of the AMP-activated protein kinase (AMPK) family. The AMPKs play major roles in regulating metabolism and cell growth [10,11,12]. Clinical studies have shown that reduced levels of SIK1 are associated with distal metastases and poor outcome in breast cancer, and SIK1 expression has been associated with a tumour suppressor function [13,14,15,16]. Cheng and co-authors [16] have demonstrated that SIK1 links the tumour suppressor Liver Kinase 1B (LKB1) to p53-dependent suppression of metastasis and that SIK1 activated by LKB1 suppresses metastasis and invasion in a human mammary epithelial cell line [16]. The LKB1-AMPK pathway is also shown to serve as a metabolic checkpoint by arresting cell growth in conditions of low intracellular ATP levels [10].

Cell migration is important in normal and pathologic gastric epithelial function [6]. Since cell migration and invasion are characteristics of the progression of gastric cancer [6], and processes known to be regulated by gastrin, we wanted to address the role of SIK1 in gastrin-mediated responses. In the present study we show that gastrin induces transient SIK1 expression in pancreatic and gastric adenocarcinoma cells, and that the gastrin-induced SIK1 expression is negatively regulated by Inducible cAMP early repressor (ICER). Ectopically expressed SIK1 increases gastrin-induced transcription of c-fos and CRE-, SRE-, AP1- and NF-kB-driven reporter plasmids. We also find that gastrin induces nuclear export of HDACs and ectopic SIK1 expression increases phosphorylation of class IIa HDAC4. Notable, the expression of MMP-9 is almost abolished in cells with ectopic expression of SIK1, indicating that the effect of SIK1...
may affect chromatin modifying events in different ways. Interestingly, we find that siRNA mediated knockdown of SIK1 enhances gastrin-induced migration of AGS-Gs cells. Collectively, this suggests a role of SIK1 in gastrin induced responses and suggest that SIK1 may act as tumour suppressor in gastric adenocarcinoma cells.

### Materials and Methods

#### Cell lines

AR42J (rat pancreatic acinar cell derived; American Type Culture Collection (ATCC), Rockville, MD) were grown in DMEM with 4.5 g/l glucose (Invitrogen), 15% fetal bovine serum (FBS; Bio Whittaker, Lonza Belgium), 1 mM sodium pyruvate, 0.1 mg/ml L-glutamine (Invitrogen), 10 U/ml penicillin-streptomycin (Invitrogen) and 1 μg/ml fungizone (Invitrogen). AGS-Gs cells (human gastric adenocarcinoma, stably transfected with CCK2 receptor [17,18]; gift from Prof. Andrea Varro, University of Liverpool, England) were maintained in Ham’s F-12 (Invitrogen, Carlsbad, CA) with 10% FBS, 10 U/ml penicillin-streptomycin and 2 μg/ml puromycin (Sigma-Aldrich, St. Louis, MO). MKN45 cells (human gastric adenocarcinoma; gift from Prof. Sue Watson, University of Nottingham) were grown in DMEM with 4.5 g/l glucose, 10% FCS, 10 U/ml penicillin-streptomycin and 1 μg/ml fungizone.

#### Transient transfection and gastrin treatment of cells

AGS-Gs cells (5.0 x 10⁵/well) were seeded in six-well plates and transfected after 24 h with 2.5 μg plasmid and 12.5 μl Metafectene PRO transfection reagent (Biontex Laboratories GmbH, Martinsried, Germany) per well. 24 h after transfection, cells were serum starved for 24 h and then treated with 5 nM gastrin for 4 h. siRNA-All-ICER (Qiagen, Germantown, MD) were annealed at 20 nM in 1.0 ml siRNA suspension buffer (Qiagen). siRNA-All-ICER: 5’-CAUUAUGCCUGUAAACUGATT-3’. siRNA towards enhanced green fluorescent protein (EGFP) [22]: 5’-GCAAGCUGACCCUGAGUU-3’.

#### Reporter gene assay

Cells (1.2 x 10⁵/well) were seeded in 96-well plates and transfected 24 h later. The cells were transfected with 84 ng plasmid, 1.68 ng (1:50) phRL-null (Promega, Madison, WI) as internal control, and 0.4 μl Metafectene PRO per well for 24 h, serum starved for 24 h and then treated with gastrin for 4 h. Luciferase activity was measured with Wallac 1420 Victor 3 plate reader (Perkin Elmer, Boston, MA) using the Dual Luciferase Reporter Assay System (Promega).

#### Immunocytochemical staining and confocal microscopy

Cells (2.0 x 10⁴/well in 200 μl medium with 10% FBS) were seeded on Lab-Tek Chambered Coverglass with 8 wells (NUNC, Thermo Scientific, Rockford, IL) and transfected with pEGFP-SIK1. After cultivation for 24 h, cells were serum starved for 24 h and then treated with 5 nM gastrin for 0–60 min. Living cells were examined by confocal microscopy. To examine endogenous CRTC2 and HDACs, cells were fixed (4% paraformaldehyde in PBS) for 10 min, washed (PBS x 2) and permeabilized (1% MeOH) for 10 min on ice and washed (PBS x 2). Further, cells were blocked for 30 min (3% goat serum in PBS), incubated with the primary rabbit anti-human CRTC2 antibody (1:100) or rabbit CRTC2 and HDACs, cells were fixed (4% paraformaldehyde in PBS) for 10 min, washed (PBS x 2) and permeabilized (Ice-cold MeOH) for 10 min on ice and washed (PBS x 2). Confocal microscopy studies were performed with a Zeiss Axiovert 100-M inverted microscope equipped with an LSM 510 laser-scanning unit and a 1.4 numerical aperture x63 Plan-Apochromat oil immersion objective. To examine living cells, the cell chamber was heated to 37°C using a Tempcontrol Digital 37-
Figure 1. Gastrin-induced activation of SIK1. A: AR42J cells were treated with gastrin and mRNA levels measured by qRT-PCR. Mean expression level relative to untreated cells is shown. Results show one representative of three independent biological experiments; mean ± SD of three technical replicates.

B: SIK1 Western blot of gastrin treated AR42J cells. A representative image is shown and quantified.

C: AGS-GR cells were treated with gastrin and mRNA levels measured by qRT-PCR. Mean ± SEM of three independent biological experiments is shown.

D: SIK1 Western blot of gastrin treated AGS-GR cells. A representative image is shown and quantified.

E: MKN45 cells were treated with gastrin and mRNA levels measured by qRT-PCR. Mean ± SEM of three independent biological experiments is shown.

F: Immunofluorescence images of gastrin treated cells showing the expression of CRTC2 and DNA. Untreated, Gastrin 30 min, and Gastrin 60 min conditions are compared.

G: Immunofluorescence images of gastrin treated cells showing the expression of pEGFP-SIK1.
to minimize photobleaching, laser power was typically 20% under maximum, and the pinhole was set to 0.8–1.2. Multitracking was used for dual colour imaging. The Zeiss LSM Image browser version 4 was used for acquisition, and processing was completed using Adobe Illustrator CS5.

Western blotting

Cells (5.0×10⁵/well) in six-well plates were harvested in 0.5 ml SDS-sample buffer and subjected to western blot analysis as described previously [23]. Binding of secondary antibodies was visualized by the Super Signal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) and Kodak Image Station 2000R (Kodak, Pittsburgh, PA). Band intensities were quantified using Adobe Photoshop Elements 8.0, and the intensities for the protein of interest were normalized to β actin intensities. The following antibodies were used: mouse monoclonal to beta actin (1:2000; Abcam, Cambridge, MA), HRP-conjugated goat anti-mouse IgG (1:1000; Cell Signaling, Beverly, MA), rabbit anti-human phospho-HDAC4 (Ser-632)/HDAC5 (Ser-498)/HDAC7 (Ser-486) antibody (1:1000; Cell Signaling), rabbit anti-human phospho-LKB1 (Ser-428) (1:1000; Cell Signaling) and Rabbit anti-human phospho Thr-182 SIK1 (1:500; generously provided by Dr H. Takemori (National Institute of Biomedical Innovation, Osaka, Japan)).

Migration assay

The xCELLigence DP system (Roche Diagnostics GmbH, Germany) was used for measurement of migration. This system utilizes specialized culture plates containing gold electrode arrays beneath the bottom of individual wells. Cellular contact with the electrode surfaces increases the impedance across these gold arrays. This impedance value is measured by the DP system and...
Figure 3. SIK1 enhances gastrin-induced transcription. AGS-GR cells cotransfected with expression plasmids and reporter plasmids and treated with gastrin (4 h). Results show one representative of three independent experiments; mean ± SD of six technical replicates. 

A: Cells co-transfected with c-fos-luc and pRES (control) or pSIK1. 

B: The effect of pSIK1 wt and pSIK1 mutants on c-fos-luc expression.

C: Cells co-transfected with CRE-luc and pRES or pSIK1.

D: The effect of pSIK1 wt and pSIK1 mutants on CRE-luc expression.

E: AGS-GR

F: MKN45

G: Endogenous HDACs

H: HDAC5 overexpression

Figure 3. SIK1 enhances gastrin-induced transcription. AGS-GR cells cotransfected with expression plasmids and reporter plasmids and treated with gastrin (4 h). Results show one representative of three independent experiments; mean ± SD of six technical replicates. A: Cells co-transfected with c-fos-luc and pRES (control) or pSIK1. B: The effect of pSIK1 wt and pSIK1 mutants on c-fos-luc expression. C: Cells co-transfected
SIK1 is induced by gastrin and inhibits migration

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Results

Gastrin induces SIK1 expression

Microarray mRNA profiling of gastrin-treated pancreas derived adenocarcinoma cells (AR42J) demonstrated that gastrin transiently activates SIK1 gene expression (Accession number: GSE32869). In the present study we further explored the role of SIK1 in the gastrin responsive cell lines AGS-G_R, MKN45 and AR42J. Quantitative RT-PCR analyses confirmed that gastrin induces a rapid, transient increase of SIK1 mRNA in AR42J cells. The SIK1 mRNA increased approximately six fold upon gastrin treatment and we observed a small but reproducible enhancement of protein expression after 2 hrs (Figure 1A–B). In gastric adenocarcinoma AGS-G_R cells, the SIK1 mRNA was peaking at 1 h (Figure 1C). SIK1 protein accumulates 4–6 h after gastrin treatment (Figure 1D), and returns to basal level after 24 h (Figure 1E). We also found that gastrin treatment of MKN45 cells resulted in enhanced SIK1 protein expression (Figure 1E).

The SIK1 promoter contains two cAMP responsive elements (CRE) and is transcriptionally regulated via the cAMP-CREB-CRTC2 pathway [24]. SIK1 protein itself regulates CRTC2 (CREB regulated transcription coactivator 2/TORC2) activity by phosphorylation of CRTC2 Ser-171 leading to its translocation to the cytoplasm with a subsequent inactivation of CREB [24,25]. It is demonstrated that SIK1 plays a role in negative feedback of CREB/CRTC2 mediated transcription of steroidogenic enzymes [26]. To investigate whether SIK1 participates in regulation of the CRTC2-CREB axis also in gastric cancer cells, we examined the subcellular localization of CRTC2 protein upon gastrin treatment in AGS-G_R cells. We did not observe any evident translocation of CRTC2 proteins from nucleus to cytoplasm (Figure 1F), indicating that gastrin treatment does not affect shuttling of CRTC2 proteins and most likely does not modulate CREB activity via this pathway in AGS-G_R. Since SIK1 has been reported to be transported out of the nucleus upon phosphorylation on Ser-577 [27], we also examined if gastrin treatment induced nuclear-cytoplasmic shuttling of SIK1 by use of EGFP-SIK1 fusion proteins. We found that EGFP-SIK1 displayed a punctual nuclear and cytoplasmic localization (Figure 1G), whereas no translocation was observed upon gastrin treatment.

ICER is a negative regulator of gastrin-induced SIK1 expression

Our result in Figure 1A–E shows that gastrin transiently induces SIK1 mRNA and protein in gastrin responsive cells. This indicates that gastrin-induced SIK1 expression might be under negative control of a gastrin-induced repressor. Results from our genomewide time series experiments in AR42J cells revealed that SIK1 is a primary gastrin-induced gene [4]. Treating AR42J cells with cycloheximide (CHX) (to block de novo protein synthesis) did not inhibit SIK1 mRNA induction by gastrin (Figure S1B and [4]), suggesting that SIK1 is a direct target gene of gastrin signalling. Gastrin induced higher levels of SIK1 in presence of CHX, supporting the assumption that SIK1 expression might be under negative control of a gastrin-induced repressor. We have previously shown that the transcriptional repressor ICER, which negatively regulates gene expression via binding to CRE promoter elements, is induced by gastrin [28,29]. Since the SIK1 promoter contains consensus CRE-binding sites [30], SIK1 is a potential ICER target gene. We found that gastrin induces ICER expression in AGS-G_R cells (Figure 2A) and hypothesized that ICER might be involved in negative regulation of SIK1 expression. Ectopic expression of the ICER splice variants ICER I or ICER II in AGS-G_R cells resulted in reduced levels of gastrin-induced SIK1 gene expression (Figure 2B). To corroborate that the repressing effect was caused by ICER, SIK1 expression was measured in presence of siRNA targeting ICER. Our results showed that siICER increases gastrin-induced SIK1 expression at the mRNA level (Figure 2C), followed by enhancement of its protein level (Figure 2D), indicating that inhibition of gastrin-induced ICER expression can de-repress SIK1 expression. Taken together, we demonstrate that ICER acts as a negative regulator of gastrin-induced SIK1 expression.

SIK1 promotes gastrin-induced transcription likely via phosphorylation of HDACs

Since SIK1 is an early gastrin responsive gene, it was of interest to unravel if SIK1 was involved in downstream transcriptional activation. We and others have previously shown that c-fos is activated by gastrin [23,31,32], and we thus wanted to explore a possible role of SIK1 in gastrin-induced c-fos activation. AGS-G_R cells with ectopically expressed SIK1 showed a two-fold increase in gastrin-induced c-fos-luc transcription compared to control cells (Figure 3A).

We further determined the gastrin response in cells with ectopic expression of the mutants SIK1 K366M [kinase dead [26]] or SIK S577A (cannot be phosphorylated at Ser-577, resulting in SIK1 nuclear sequestration [27]). SIK1 K366M elicits significantly lower gastrin-induced activation of c-fos-luc compared to SIK1 wt (Figure 3B), supporting our anticipation that the kinase activity of SIK1 is important for the enhancing effect of gastrin-induced c-fos transcription. Ectopically expressed SIK1 S577A, expected to be localized exclusively in the nucleus [27], results in approximately
Figure 4. SIK1 inhibits migration in AGS-Gr cells via suppression of MMP-9. A–B: AGS-Gr cells (A) and MKN45 cells (B) were treated with gastrin, and phospho-LKB1 (Ser-428) protein levels determined by Western blot. The phospho-LKB1 bands from a representative experiment are shown.

C–D: AGS-Gr cells (C) and MKN45 (D) were treated with gastrin, and phospho-SIK1 (Thr-182) protein levels determined by Western blot. The phospho-SIK1 bands from a representative experiment are shown.

E: AGS-Gr cells transfected with siSIK1 or siCtr and real-time cell migration monitored (0–24 h). Results show one representative of three independent experiments (mean ± SD of three technical replicates).

F: MMP-9 mRNA expression in cells transfected with pSIK1 and treated with gastrin. Results show one representative of three independent experiments, (mean ± SD). doi:10.1371/journal.pone.0112485.g004
phosphorylated HDAC4. In AGS-GR cells, the SIK1-induced SIK1 leads to a weak but reproducible enhancement of cells with ectopic expression of SIK1. As shown in Figure 3E–F, SIK1 enhances gastrin-induced transcription via a general mechanism targeted by different transcription factors, suggesting that SIK1 of SIK1 on gastrin-mediated transcriptional activation via this large variety of promoter elements, each of which is known to be phosphorylation of HDAC leads to cytosolic translocation and activation of transcription. In the gastric adenocarcinoma cell line AGS-GR ectopic SIK1 inhibits migration.

2.5 fold higher gastrin-induced c-fos activation compared to SIK1 wt (Figure 3B). Our results may indicate that SIK1 promotes gastrin-induced c-fos transcription by phosphorylating a nuclear target.

The c-fos promoter contains several transcription factor binding sites including CRE, SRE and AP1 [33]. To further identify promoter elements involved in SIK1-mediated transcriptional regulation, we examined the effect of ectopic SIK1 expression on gastrin-induced CRE-, SRE-, AP1- and NF-κB-driven luciferase reporter plasmids. The results show that SIK1 expression enhances gastrin-mediated transcription via CRE (Figure 3C), SRE, AP1 and NF-κB promoter elements (Figure 3D). The effect of SIK1 on gastrin-mediated transcriptional activation via this large variety of promoter elements, each of which is known to be targeted by different transcription factors, suggests that SIK1 enhances gastrin-induced transcription via a general mechanism (e.g. modifying chromatin) that may affect several specific promoter-transcription factor interactions.

Histone deacetylases (HDAC) are enzymes modifying histones by removing acetyl groups on the histones. Class IIA HDACs (HDAC 4/5/7/9) are recruited to specific promoters by sequence-specific DNA-binding proteins, with a subsequent deacetylation of local chromatin resulting in repression of transcription (reviewed in [34]). More recently, SIK1 was shown to phosphorylate class IIA HDACs, leading to their translocation to cytoplasm and repression of gene expression [30,35,36]. We hypothesized that phosphorylation of HDAC may represent a general mechanism underlying the enhancement of transcription that we observed in our reporter gene studies. We therefore investigated gastrin-induced HDAC4/5/7 phosphorylation in AGS-GR and MKN45 cells with ectopic expression of SIK1. As shown in Figure 3E–F, gastrin induces phosphorylation of HDAC4 and overexpression of SIK1 leads to a weak but reproducible enhancement of phosphorylated HDAC4. In AGS-GR cells, the SIK1- induced HDAC4 phosphorylation is observable already after 15 min of gastrin-stimulation and is still markedly enhanced after 2 h. In MKN45 cells the gastrin induced phosphorylation of HDAC4 was observed from 10 to 60 min (Figure 3F). We also show that gastrin phosphorylation of LKB1 at Ser-428, an amino acid residue shown to be involved in increased apoptosis in endothelial cells [38]. We also find that gastrin mediates SIK (Thr-182) phosphorylation in both cell lines (Figure 4C–D). Taken together, this suggests that gastrin-induced LKB1 phosphorylation is responsible for the subsequent SIK1 activation.

In cancer biology SIK1 expression has been associated with a tumour suppressor function [13,14,15,16]. Cell migration is an important feature of oncogenesis, and gastrin is shown to induce migration in AGS-GR cells [5]. Thus we proceeded to examine the role of SIK1 in gastrin-induced migration. AGS-GR cells, which have the ability to migrate in vitro were transfected with siSIK1/+.– gastrin and migration assessed using real-time cell monitoring assay (xCELLigence technology). We show that cells with SIK1 knockdown display significantly higher migratory activity compared to control cells (transfected with Silencer Negative Control siRNA [sCtrl]), and that this difference is enhanced in gastrin-treated cells (Figure 4E), suggesting that SIK1 participates in migration-repressing processes in gastric cancer cells.

Several members of the matrix metalloproteinase (MMP) family are shown to trigger cancer cell migration, so we hypothesized that the inhibitory effect of SIK1 on migration may involve regulation of MMPs. Since MMP-9 is one of several extracellular proteins reported to participate in gastrin-mediated regulation of invasion [6], we hypothesized that the inhibitory effect of SIK1 on migration may involve regulation of MMP-9. AGS-GR cells were transfected with pSIK1 and treated with gastrin (0–6 h). We found that ectopically expressed SIK1 reduced the gastrin induced MMP-9 expression (Figure 4F). Our results suggest that the SIK1-mediated reduction of gastrin-induced migration could in part be explained by reduced levels of the migration enhancing protein MMP-9.

**Discussion**

Several studies have reported that α- and β-adrenergic receptors and Ser/Thr kinase receptors mediate regulation of SIK1 [27,40,41,42]. In the present study we show that gastrin-mediated signalling, known to involve the Gq/G12-coupled CCK2 receptor and signalling pathways like PEI-KPK/Akt, PKC and MEK-ERK1/2 [29,31,43], induces transient SIK1 expression in adenocarcinoma cells. Gastrin treatment did not give any evident translocation of ectopically expressed SIK1 proteins nor endogenous CRTC2, suggesting that the well-established SIK1/CRTC2/CREB axis [25] does not occur or play any prominent role in gastrin mediated signalling.
We identified SIK1 as a primary gastrin-responsive gene, negatively regulated by the transcriptional repressor ICER. We have previously shown that ICER is involved in down-regulation of gastrin-induced genes like Cyclin D1 and e-fos [23]. The observations in the current study thus support the hypothesis that induction of HDACs in the gastric epithelium is part of an intracellular feedback mechanism contributing to the attenuation of biological responses to gastrin.

We demonstrate that ectopically expressed SIK1 enhances gastrin-induced phosphorylation of HDAC4 (illustrated in Figure 5), confirming the role of SIK1 as a class Ia HDAC kinase, as described in other cell systems [30,35,44]. However, the role of gastrin in mediating HDAC phosphorylation and export has not been shown earlier. The class Ia HDACs (HDAC4/5/7/9) are thought to act as transcriptional corepressors by deacetylating nucleosomal histones. Class Iia HDAC-mediated repression is relieved by phosphorylation which results in their cytoplasmic accumulation, sequestering them from histone substrates and renders them enzymatically inactive [reviewed in [34]]. SIK1 phosphorylation of HDAC4 might be the mechanism underlying our observed SIK1-mediated enhancement of gastrin-induced transcription. This coincides with the findings of Pan et al [45] who reported that HDAC4 represses e-fos transcription, and that phosphorylation of HDAC4 will de-repress the transcription [45]. Others have shown that the CREB/ATF binding site in the mouse c-fos promoter is subject to transcriptional repression via chromatin remodelling mechanisms [46,47]. Hence, we propose that SIK1, upon gastrin stimulation, mediates phosphorylation of HDACs, induces nuclear export and thereby relieving HDAC-induced repression of c-fos transcription. Since our results included SIK1 elicited enhancement of transcription via a variety of promoter elements (CRE, SRE, AP1, NF-kB), we suggest that SIK1-mediated phosphorylations and subsequent inactivation of HDACs may enhance the gastrin-mediated transcription of a large number of genes, as also reported by others [30,44].

We show that SIK1 knockdown by siRNA brings along higher migratory activity in gastric adenocarcinoma cells, indicating that SIK1 suppresses gastrin-mediated migration. Our observation that LKB1 Ser-428 and SIK1 Thr-182 are phosphorylated upon SIK1 suppresses gastrin-induced migration. To conclude, in this study we characterize a function of SIK1 in gastric adenocarcinoma cells. We show that SIK1 is regulated by gastrin and that SIK1 inhibits gastrin induced migration.

Supporting Information

Figure S1 A: SIK1 Western blot of gastrin treated AGS-GR cells. A representative image is shown. The SIK1 bands from the experiment are quantified. B: Microarray gene expression analysis showing the effect of the protein synthesis inhibitor cycloheximide (CHX) on gastrin-induced SIK1 mRNA expression in AR42 cells. Cells were pre-treated with CHX (10 μg/ml) for 30 min before gastrin (10 nM) were added. Expression level relative to time point zero is shown. (EPS)

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Author Contributions

Conceived and designed the experiments: LKMS LT. Performed the experiments: LKMS SR TSS IH KM WSP. Analyzed the data: LKMS TB. Contributed to the writing of the manuscript: LKMS LT AL TB.

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