Loss of RACK1 Promotes Metastasis of Gastric Cancer by Inducing a miR-302c/IL8 Signaling Loop

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Abstract

Gastric cancer remains the third leading cause of cancer-related mortality worldwide, and invasion and metastasis of gastric cancer represent the major reason for its poor prognosis. In this study, we found that loss of the receptor for activated C-kinase 1 (RACK1) promoted the metastasis of gastric cancer by enhancing the autocrine of IL8 in vitro and in vivo. microRNA (miRNA; miR) array identified that RACK1 modulated the expression of a series of miRNAs, including the miR-302 cluster, and RACK1 modulated the IL8 expression and tumor invasion through miRNA-302c. Moreover, upregulation of IL8 in turn decreased the level of miRNA-302c and induced IL8 expression in a feedback manner. Tissue microarray also indicated that RACK1 was correlated with invasion/metastasis phenotype, IL8 expression, as well as 5-year survival in clinical cases of gastric cancer. Together, our results imply that loss of RACK1 in gastric cancer links epigenetics to inflammatory cytokines to promote tumor metastasis. Cancer Res; 75(18); 3832-41. ©2015 AACR.

Introduction

Gastric cancer remains the third leading cause of cancer-related mortality worldwide, although its incidence has decreased over the past six decades (1, 2). Most patients are diagnosed with gastric cancer at advanced stage, when the 5-year survival rate ranges only from 4% to 20% for surgically resected cases (3). Invasion and metastasis of gastric cancer represent the major reason for its poor prognosis. It was reported that lymph node metastasis presented in more than 50% of gastric cancer patients when they were initially diagnosed (4), whereas peritoneum metastasis might be already present in 5% to 20% of patients undergoing gastric resection in curative intent (5). Even if the curative resection was possible, the recurrence could occur in approximately 60% of patients, and the median survival rates for recurrent gastric cancer patients range only from 9 to 14 months (6).

It is increasingly apparent that the soluble factors in tumorigenic microenvironment is involved in the acquired capability for invasive growth and metastasis (7). Multiple proinflammatory cytokines, chemokines, and growth factors, such as IL1β, IL8, and IL17, could be secreted either by the tumor cells themselves or by any of the various cellular components of the tumor microenvironment (8). In particular, tumor-derived cytokines have been shown to function in an autocrine way promoting tumor growth, survival, and acquisition of metastatic potential (9), as well as to work in a paracrine fashion to reprogram the normal stroma to a tumorigenic stroma (10). Thus, it appears that an altered epithelium itself alters the mediators that ultimately create a tumorigenic microenvironment (11). Increased expression of the proinflammatory CXC chemokine IL8 has been characterized in various cancer cells, endothelial cells, infiltrating neutrophils, and tumor-associated macrophages, and IL8 functions as an important tumorigenic factor within the tumor microenvironment through binding to cell-surface G protein–coupled receptors CXCR1 and CXCR2 (12, 13).

RACK1 is a member of the tryptophan–aspartate repeat (WD-repeat) family of proteins, and adopts a seven-bladed propeller structure that facilitates protein binding (14). RACK1 has been identified as a classic scaffold protein for multiple kinases and receptors, and plays a pivotal role in a wide range of biologic responses, including signal transduction, as well as cell growth, migration, and differentiation (15). The dysregulation of RACK1 is reported in several kinds of cancers, and has dramatic consequences on the regulation of key signaling pathways, and therefore on the development and progression of neoplastic diseases (14). Our previous study also revealed that ribosomal RACK1 promoted the chemoresistance and growth in hepatocellular carcinoma (16). Although the mechanisms by which...
RACK1 modulates the progression of cancer may be multifaceted (14), the exact role of RACK1 in gastric cancer metastasis remains little understood. In this study, we found that RACK1 was associated with the invasion and metastasis of gastric cancer by tissue microarray, and RACK1 suppressed invasiveness in gastric cancer by modulating the microRNA (miR)-302c/IL8 axis in vitro and in vivo.

Materials and Methods

Patient samples

For tissue microarray detection, tumor specimens were obtained from 495 gastric cancer patients who underwent surgical resection without preoperative treatment from February 2000 to July 2008, at Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China. Their age ranged from 30 to 86 years (mean, 61.39 ± 11.39 years). The diagnosis of gastric carcinoma was confirmed by pathologic examination. Staging data were according to the seventh edition of the AJCC Cancer Staging Manual (3). All the patients’ demographic characteristics, date of surgery, tumor stage, surgical and medical treatment methods, survival time, and other relevant data were extracted from hospital records. The independent group of 60 gastric cancer samples was also collected at Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China. The use of human tissue samples and clinical data was approved by the ethics committee of the Fudan University. All donors were informed of the aim of the study and gave consent to donate their samples.

Cells and reagents

Human gastric cancer cell lines, HGC-27, MGC80-3, AGS, NCI-N87, SGC-7901, and BGC-823, were purchased from Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences. All cell lines obtained from the cell bank are tested for authentication using short tandem repeat fingerprinting and passaged for fewer than 6 months. Cells were cultured in RPMI-1640 or DMEM supplemented with 10% fetal bovine serum (FBS; cat. no. 16000-044; Gibco; Supplementary Table S5) at 37°C in a humidified atmosphere containing 5% CO2. For conditioned medium (CM) collection, cell-normalized (1 × 106 cells/mL for 24 hours) serum-free supernatants were collected.

Neutralizing IL8 antibody (cat. no. MAB208) and recombinant human IL8 (rhIL8; cat. no. 208-IL) were purchased from R&D Systems. The Wnt/β-catenin pathway–specific inhibitor XAV-939 (cat. no. X3004) was purchased from Sigma–Aldrich.

Plasmids

The human RACK1 cDNA was in frame subcloned into pcDNA3.1-Myc/His vector (Invitrogen) as previously described (16), and was a gift from Jean-Luc Parent (Université de Sherbrooke, Sherbrooke, Québec, Canada). Wild-type il-8 3′-LTR sequence, which was a gift from Dr. Richard G. Pestell (Thomas Jefferson University and Hospital, Kimmel Cancer Center, Philadelphia, PA), was inserted into the XbaI–FseI site immediately downstream of the stop codon in the pGL3 control firefly luciferase reporter vector (Promega; ref. 17). The vector containing mutated il-8 3′-LTR was constructed using QuickChange Lightning Mutagenesis Kit (Stratagene; cat. no. 200522) following the manufacturer’s instructions.

Animal studies

Animal experiments were performed according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the NIH, and also approved by the ethics committee of the Fudan University. Four- to 6-week-old male BALB/c nude mice were obtained from Shanghai Laboratory Animal Center of Chinese Academy Sciences and housed in a pathogen-free room. Orthotopic gastric cancer models were developed as described previously (18). Briefly, 1 × 107 stable gastric cancer cells (HGC-27-shScr or HGC-27-shRACK1) in 100 μL of PBS were injected subcutaneously into the flank of nude mice. Subsequently, subcutaneous tumors were removed and minced into pieces. Then, a tumor piece was fixed to the site of the serosal surface in the greater curvature of the glandular stomach of nude mice. Then, orthotopic transplanted mice were randomly divided into two groups separately. Ten mice of each group were given IgG (4 mg/kg) or IL8 neutralizing antibody (4 mg/kg) by i.p. injection, or administrated with vehicle or Remipressin (10 mg/kg) by i.p. injection. Tumor invasion, liver infiltration, and peritoneal metastasis were examined 6 weeks after implantation.

Statistical analysis

Results are presented as mean ± SD. CES analysis was performed with nonparametric methods. The optimal cutoff value of CES is determined by ROC curve analysis. Categorical data were analyzed using the χ2 test. Correlation of RACK1 with IL8 or miR-302–cluster expression was analyzed using the nonparametric Spearman ρ test. The Kaplan–Meier method was used to determine survival probability and differences were assessed by the log-rank test. Statistical significance was set at two-sided P < 0.05. All analysis was performed using SPSS 13.0 software.

Results

RACK1 Inhibits Gastric Cancer Metastasis

Invasion and metastasis of gastric cancer represent the major reason for its poor prognosis. To understand whether RACK1 modulated invasion and metastasis of gastric cancer, we first evaluated the effect of RACK1 shRNA on the invasion of gastric cancer cells in vitro (Supplementary Fig. S1). Transwell assays demonstrated that knockdown of RACK1 promoted the invasiveness of gastric cancer cells in vitro (Fig. 1A). Moreover, treatment with conditioned medium obtained from RACK1-silenced cells significantly induced the invasiveness of gastric cancer cells, suggesting that downregulation of RACK1 influenced the invasiveness of gastric cancer cells in an autocrine-dependent manner (Fig. 1B). We therefore assessed the global effect of RACK1 silencing on the pattern of cytokines using cytokine antibody arrays. Among the relatively highly expressed cytokines, IL8 was increased significantly and consistently (Fig. 1C and Supplementary Fig. S2). Real-time PCR and ELISA also confirmed the upregulated mRNA level and secretion of IL8 upon RACK1 silencing, respectively (Fig. 1D). The mRNA levels of RACK1 and IL8 were negatively correlated in all gastric cancer cell lines, whereas no statistical significance was detected possibly due to the limited sample size (Fig. 1E). We also evaluated the expression of IL8 receptor CXCR1/2 in gastric cancer cells. Flow cytometry analysis revealed that CXCR1 and
CXCR2 were differentially expressed on the surface of gastric cancer cell lines at different levels (Supplementary Fig. S3A). Moreover, depletion of RACK1 showed little effect on the mRNA expression of CXCR1/2 in all gastric cancer cell lines (Supplementary Fig. S3B).

It is known that increased IL8 levels in gastric cancer is correlated with the depth of invasion, venous infiltration, and lymphatic invasion (19). We next explored the role of IL8 in RACK1-mediated modulation of invasiveness in gastric cancer cells. As shown in Fig. 1F and Supplementary Fig. S4A, administration of IL8-neutralizing antibody blocked RACK1 depletion-induced invasion of gastric cancer cells in vitro. Moreover, RACK1 overexpression suppressed the invasion of gastric cancer cells in vitro, and additional administration of recombinant IL8 restored the invasiveness in RACK1-overexpressed gastric cancer cells (Fig. 1G and Supplementary Fig. S4B). These results suggest that RACK1 suppresses the invasion of gastric cancer cells by modulating the autocrine of IL8 in vitro.

RACK1 suppresses the metastasis of gastric cancer in IL8-dependent manner in vivo

Because mice do not have the IL8 gene, human cancer cell lines and xenograft studies have been used to study the role of IL8 in many human cancers, including gastric cancer (20). We next established the orthotopic gastric cancer models to investigate the modulatory effect of RACK1, as well as the active role of IL8, for metastasis in vivo (Fig. 2A). Although faint IL8 staining was found in control tumor xenograft, human IL8 could be detected in plasma at the level of approximately 27.27 pg/mL probably due to the secretion of this chemokine from tumor cells into tumor microenvironment and blood (Fig. 2B and C), and IL8 expression was remarkably increased in primary tumor sections and plasma.
of mice bearing RACK1-silenced cells (Fig. 2B and C). Moreover, knockdown of RACK1 also significantly enhanced tumor invasion into muscularis propria, the incidence of liver infiltration as well as numbers of peritoneal metastatic nodules. Additional administration of IL8-neutralizing antibody or CXCR1/2 antagonist Reparixin effectively restrained tumor local dissemination and peritoneal metastasis induced by RACK1 shRNA (Fig. 2D and F), suggesting that tumor-derived IL8 was essential for RACK1 depletion-induced tumor invasion and metastasis in vivo. Meanwhile, neutralizing IL8 or Reparixin also significantly improved overall survival of mice in both control (shScr) and RACK1-silenced groups, and suppressed RACK1 silencing-induced shortened survival (Fig. 2E and G). These results suggest that loss of RACK1 enhances the invasion and metastasis of gastric cancer in an IL8-dependent manner in vivo.

miR-302 cluster is regulated by RACK1 and targets IL8 in gastric cancer

We next determined how RACK1 regulated the expression of IL8 in gastric cancer. Because previous studies have suggested that RACK1 negatively regulates Wnt signaling in gastric cancer (21), and IL8 might be a target of the Wnt/β-catenin pathway (22), we employed XAV-939, a specific inhibitor of the Wnt/β-catenin pathway (23), in RACK1-silenced cells. However, treatment of XAV-939 showed little effect on RACK1 depletion-induced IL8 upregulation (Supplementary Fig. 5S), suggesting that the Wnt/β-catenin pathway does not contribute to RACK1-mediated regulation of IL8 expression in gastric cancer.

It has been reported several kinds of inflammatory cytokines, including IL8 (24), are regulated by microRNAs (miRNA; miR) at posttranscription level (25). RACK1 has also been shown to be associated with miRNA function in mammals (26). We next explored whether RACK1 regulated expression of IL8 via certain miRNAs. As showed in Fig. 3A, miRNA expression profiling in control and RACK1-silenced gastric cancer cells was carried out using microarrays. Among the significantly regulated miRNAs upon RACK1 silencing, the miR-302 cluster, including miR-302a/b/c/d, was downregulated and could potentially bind to the il-8 3′-UTR by bioinformatical analysis (Fig. 3E). Real-time PCR analysis confirmed that RACK1 depletion suppressed the expression of the miR-302 cluster, and miR-302a/b could not be detected in most gastric cancer cell lines (Fig. 3B and Supplementary Fig. 6S). The positive correlation between the RACK1 mRNA level and the miR-302 cluster was also confirmed in gastric cancer cell lines and clinical tumor tissues (Fig. 3C and D).
We next examined the potential role of the miR-302 cluster in modulating IL8 expression in gastric cancer cells. A luciferase activity assay also confirmed that the miR-302 cluster targeted the 3′-UTR of IL8 mRNA (Fig. 3F and Supplementary Fig. S7A and S7B). Moreover, transfection of miR-302c or miR-302d mimics suppressed the expression of IL8, both at mRNA level and in the supernatant (Fig. 3G and Supplementary Fig. S7C), while inhibition of miR-302c induced upregulation of IL8 in gastric cancer cells (Supplementary Fig. S7D). However, miR-302d inhibitor showed little effect on IL8 expression (Supplementary Fig. S7D), which might be attributed to the much higher level of endogenous miR-302c than miR-302d in gastric cancer cells (Supplementary Fig. S8). These results imply that IL8 mRNA is the target of the miR-302 cluster, and miR-302c is the predominant member of the miR-302 cluster in gastric cancer.

We also examined the potential effects of RACK1 on the expression of miRNAs that had been reported to target IL8 mRNA, including the miR-17, miR-20a, miR-93, miR-141, and miR-200 cluster (17, 27, 28). A real-time PCR assay revealed that depletion of RACK1 induced upregulation of miR-17 and miR-20a, while inhibition of miR-302c induced downregulation of miR-200 (Fig. 3A and B). These results suggest that RACK1 regulates the expression of miRNAs that target IL8 mRNA in gastric cancer cells.
and miR-93, while the miR-141/200 cluster could hardly be detected in HGC-27 and MGC80-3 cells (Supplementary Fig. S9A). However, RACK1 shRNA suppressed the expression of miR-141 and miR-200a/b/c in AGS cells (Supplementary Fig. S9A). Further analysis demonstrated that endogenous levels of miR-141 and miR-200a/b/c were much lower than that of miR-302c, and inhibition of the miR-141/200 cluster failed to increase IL8 expression in AGS cells (Supplementary Fig. S9B and S9C). Therefore, miR-302c might be the most predominant IL8-targeting miRNA that is regulated by RACK1 in gastric cancer cells.

We next evaluated whether miR-302 modulated the invasion of gastric cancer cells via IL8. As shown in Fig. 4A, transfection of miR-302c or miR-302d mimics suppressed the invasion of tumor cells, and additional administration of recombinant IL8 restored IL8 expression in AGS cells (Supplementary Fig. S9B and S9C). Therefore, miR-302c might be the most predominant IL8-targeting miRNA that is regulated by RACK1 in gastric cancer cells.

We next evaluated whether miR-302 modulated the invasion of gastric cancer cells via IL8. As shown in Fig. 4A, transfection of miR-302c or miR-302d mimics suppressed the invasion of tumor cells, and additional administration of recombinant IL8 restored
RACK1 suppresses IL8 autocrine and restrains invasiveness through modulating miR-302c in gastric cancer

We next examined the potential role of miR-302 in RACK1-mediated regulation of IL8 expression and tumor invasion in gastric cancer cells. Transfection of miR-302c/d mimics decreased the expression of IL8 in tumor cells, and also suppressed RACK1 depletion-induced upregulation of IL8 (Fig. 4C and Supplementary Fig. S10A). Meanwhile, transfection of miR-302c inhibitor, but not miR-302d inhibitor, blocked RACK1-induced downregulation of IL8 (Fig. 4D and Supplementary Fig. S10B). Furthermore, we assessed the effects of miR-302c/d on the invasiveness of gastric cancer cells regulated by RACK1. Mimics of miR-302c and miR-302d both suppressed the invasive ability induced by RACK1 depletion (Fig. 4E and Supplementary Fig. S10C), and inhibition of miR-302c reversed the anti-invasive effect of RACK1 overexpression in gastric cancer cells (Fig. 4F and Supplementary Fig. S10D). Together, these results suggest RACK1 suppresses IL8 autocrine and restrains invasiveness primarily through modulating miR-302c in gastric cancer.

RACK1/miR-302c axis is correlated with metastasis and prognosis of gastric cancer patients

To explore whether the RACK1/miR-302c axis could influence metastasis and prognosis in clinical gastric cancer cases, a tissue microarray of gastric cancer samples was employed to examine the relationship of RACK1/miR-302c expression and clinicopathologic characteristics (Supplementary Fig. S11). As shown in Fig. S5A and Supplementary Table S1, low expression of RACK1 in gastric cancer was correlated with relatively higher invasion depth, lymph node metastasis, and late TNM stage. To better understand the role of RACK1 in gastric cancer metastasis, we also examined the expression pattern of RACK1 in primary tumor tissue and corresponding metastatic foci of lymph node. Results demonstrated that the expression of RACK1 was profoundly lower in metastatic foci of lymph node by comparing with that in corresponding primary tumor tissue via immunohistochemistry (Fig. 5B) and real-time PCR (Fig. 5C) assays, respectively. Immunohistochemical analysis also presented a strong negative correlation between RACK1 and IL8 expression in clinical gastric cancer samples (Fig. 5D). Moreover, in accordance with RACK1, low expression of miR-302c in gastric cancer was also correlated with relatively higher tumor invasion depth, lymph node metastasis, and late TNM stage in clinical samples (Supplementary Fig. S12).

We also assessed the value of RACK1 in predicting the prognosis of gastric cancer cases. Kaplan–Meier survival analysis revealed that low expression of RACK1 predicted poor prognosis for overall 5-year survival in all gastric cancer cases after surgery, and the predictive value of RACK1 for overall 5-year survival was more significant in T2 to T4 patients (Fig. 5E and Supplementary Fig. S13). Cox regression multivariate analysis also revealed that RACK1 expression level, as well as tumor invasion depth, distant metastasis, and TNM stage, were independent prognostic factors for the survival of gastric cancer patients (Supplementary Table S2). In addition, high intratumoral expression of IL8 or low level of miR-302c also predicted shorter overall 5-year survival in all gastric cancer patients (Fig. 5F and G). These results suggest that the RACK1/miR-302c axis is correlated with the metastasis and prognosis in gastric cancer patients.

Feedback effect of IL8 signaling on miR-302c/IL8 axis in gastric cancer cells

Because IL8 plays a critical role in tumorigenesis and development of gastric cancer (29), we next examined whether IL8 upregulation could in turn modulate the expression of RACK1, IL8, or miR-302 cluster. As showed in Fig. 6A, after treatment with rhIL8, mRNA levels of IL8 was elevated and miR-302c expression was decreased significantly, while little effect on expression of RACK1 or other members of the miR-302 cluster was observed. Furthermore, treatment with IL8 receptor inhibitor Reparixin resulted in remarkable downregulation of IL8 and upregulation of miR-302c (Fig. 6B). These findings suggest a feedback effect of IL8 signaling on the miR-302c/IL8 axis in gastric cancer cells, and specific downregulation of miR-302c may be involved in IL8 signaling-induced IL8 upregulation.

Discussion

RACK1 has been recognized as a classic scaffold protein that takes part in regulating multiple signaling pathways involved in tumorigenesis (14). Our recent data also indicate that RACK1 provides a platform on ribosome to allow the modulation of protein translation in hepatocellular carcinoma (16). Although a previous study implies that RACK1 negatively regulates the Wnt signaling pathway in gastric cancer (21), the role of RACK1 and the underlying mechanism in modulating the metastasis of gastric cancer remain little defined. In this study, our findings demonstrate that RACK1 suppresses metastasis of gastric cancer in vitro and in vivo, through modulating the miR-302c/IL8 axis. Our data implicate a novel mechanism by RACK1 in modulating tumor invasion (Fig. 6C), and suggest that dysregulation of RACK1 may modulate the autocrine of proinflammatory cytokines to promote the progression of gastric cancer.

IL8 is known as an inflammatory chemokine and has been proposed to contribute to the formation of tumor microenvironment and development of cancer (30). Other than its defined roles in tumor microenvironment, such as myeloid-derived suppressor cell recruitment, myofibroblast expansion, and tumor angiogenesis (11), IL8 was demonstrated recently to mobilize immature myeloid cells and, in turn, accelerate tumorigenesis (20). In this study, we identified tumor-derived IL8 as the key factor involved in RACK1-mediated regulation of gastric cancer metastasis in vitro and in vivo (Figs. 1 and 2). Evidently, these results expand the multiple roles of IL8 as an active factor in tumor microenvironment. In addition to IL8, we also found that the expression of chemokines MCP-1 and RANTES (Fig. 1C and Supplementary Fig. S2), which have been shown to mediate tumor-promoting cross-talks between tumor and tumor microenvironment (31, 32), were increased upon RACK1 silencing. Therefore, it is likely that dysregulation of RACK1 may also promote the formation of tumorigenic microenvironment in a paracrine manner to modulate the initiation and progression of gastric cancer in vivo.
Previous studies have proposed different roles of RACK1 in miRNA gene transcription, primary miRNA biogenesis, processing/stability, the loading of miRNAs into miRISCs, and the recruitment of miRISCs to the ribosome (33–35). Our results demonstrated that RACK1 modulated a series of miRNA expression, including the miR-302 cluster, in gastric cancer. Despite that its precise mechanisms of action are still unclear, RACK1 is reported to be required for proper miRNA function (26, 35). Therefore, decreased expression of RACK1 in gastric cancer might not only contribute to the suppression of the miR-302 cluster, but also attenuate miR-302-mediated decay of Il-8 mRNA. Because the miR-302 cluster is a potential stemness regulator in ESCs and tumor-initiating cells (TIC; refs. 36, 37), it is likely that RACK1/miR-302c signaling may not only contribute to the regulation of tumor invasion, but also be involved in modulating TICs self-renewal in gastric cancer.

Interestingly, we also found a feedback loop between miR-302c and Il8 signaling (Fig. 6A and B), and miR-302c was shown to be the most predominant member of the miR-302 cluster in various gastric cancer cell lines (Supplementary Fig. S8), suggesting that the transcription of miR-302c may be more active in gastric cancer cells. We assume that downregulation of RACK1 could be a potent promoter of gastric cancer metastasis by initiating the miR-302c/Il8 loop, and also be a risk factor that may predict the metastasis and prognosis in gastric cancer patients.

To date, gastric cancer remains the fifth most common cancer and the third leading cause of cancer-related mortality worldwide (1, 2). While earlier diagnosis would help to improve outcomes for patients, advanced gastric cancer continues to have poor clinical outcomes mainly because of the invasion and metastasis of the disease (38). Although the utility of classic chemotherapy agents has been explored, advances have been slow and the efficacy of...
these agents has reached a plateau. Therefore, targeted therapies, whether as single-agent therapy or in combination with traditional therapies, may yet have a potential impact on improvement of the overall prognosis of gastric cancer. Our data suggest RACK1 as a new biomarker to establish the risk and prognosis of gastric cancer and to help in the selection of therapeutic modalities in clinical practice, and propose a strategy to target RACK1 as a potential adjuvant therapy for gastric cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: L. Chen, Y. Ruan, Y. Sun, X. Qin, J. Gu Development of methodology: L. Chen Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Chen, L. Min, J. Zhao, W. Chen, Z. Shen, Z. Tang, Q. Gan Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Chen, L. Min, J. Zhao, H. Chen, W. Chen, Z. Shen, Z. Tang, Q. Gan, Y. Ruan Writing, review, and/or revision of the manuscript: L. Chen, H. Chen, Y. Ruan

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Chen, X. Wang, J. Qin

Study supervision: L. Chen, J. Qin, J. Gu

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Accession Codes

microRNA array data from gastric cancer HGC-27 and AGS cells are available in ArrayExpress via accession numbers E-MTAB-3484 and E-MTAB-2619, respectively.

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Figure 6.
Feedback effect of IL8 signaling on the miR-302c/IL8 axis in gastric cancer. A, mRNA levels of RACK1 and IL8, as well as expression of the miR-302 cluster were detected by real-time PCR upon rhIL8 (100 pg/mL) treatment in HGC-27 and MGC80-3 cells. B, mRNA levels of RACK1 and IL8, as well as expression of the miR-302 cluster were detected by real-time PCR upon Reparixin (100 nmol/L) treatment in HGC-27 and MGC80-3 cells. C, simplified schematic diagram indicates the potential role of RACK1 in regulating the invasion and metastasis in gastric cancer. In brief, RACK1 upregulates the expression of miR-302c, and thereby inhibits IL8 expression and restrains tumor invasion and metastasis. IL8 signaling also exerts a feedback effect on modulating miR-302c and IL8 expression.

** P < 0.01; NS, no significance; UD, undetectable.
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