Integrated analysis of the miRNA, gene and pathway regulatory network in gastric cancer

HAIYANG ZHANG[1*], YANJUN QU[1*], JINGJING DUAN[1*], TING DENG[1*], RUI LIU[1], LE ZHANG[1],
MING BAI[1], JIALU LI[2], LIKUN ZHOU[1], TAO NING[1], HONGLI LI[1], SHAOHUA GE[1],
HUA LI[1], GUOGUANG YING[1], DINGZHI HUANG[1] and YI BA[1]

[1]Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer,
Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060;
[2]Department of Gastroenterology, Tianjin First Center Hospital, Tianjin 300192, P.R. China

Abstract. Gastric cancer is one of the most common malignant tumors worldwide; however, the efficacy of clinical treatment is limited. MicroRNAs (miRNAs) are a class of small non-coding RNAs that have been reported to play a key role in the development of cancer. They also provide novel candidates for targeted therapy. To date, in-depth studies on the molecular mechanisms of gastric cancer involving miRNAs are still absent. We previously reported that 5 miRNAs were identified as being significantly increased in gastric cancer, and the role of these miRNAs was investigated in the present study. By using bioinformatics tools, we found that more than 4,000 unique genes are potential downstream targets of gastric cancer miRNAs, and these targets belong to the protein class of nucleic acid binding, transcription factor, enzyme modulator, transferase and receptor. Pathway mapping showed that the targets of gastric cancer miRNAs are involved in the MAPK signaling pathway, pathways in cancer, the PI3K-Akt signaling pathway, the HTLV-1 signaling pathway and Ras signaling pathway, thus regulating cell growth, differentiation, apoptosis and metastasis. Analysis of the pathways related to miRNAs may provides potential drug targets for future therapy of gastric cancer.

Correspondence to: Dr Yi Ba or Dr Dingzhi Huang, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, P.R. China
E-mail: bayi@tjmuch.com
E-mail: dingzhih72@163.com

*Contributed equally

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Introduction

Gastric cancer is the second leading cause of cancer-related mortality worldwide and is the most common malignancy in China and Japan, affecting approximately one million individuals every year (1,2). The highest ratio, up to 69 cases per 100,000 individuals per year, has been determined in males in Northeast Asia (3). Prevention and personalized treatment are regarded as the best options to reduce gastric cancer mortality rates. Gastric cancer is usually a result of a high intake of various traditional salt-preserved foods and salt, concomitant with a low consumption of fresh fruit and vegetables (4,5). Analysis of the signaling pathways in cancer cells provides novel biomarkers for diagnosis and drug targets for treatment.

miRNAs are a novel class of small non-coding RNAs, typically 22 nucleotides in length (6). miRNAs suppress gene expression by directly binding to mRNAs, causing translation repression or mRNA cleavage (7,8). They are single-stranded RNAs that negatively regulate gene expression at the post-transcriptional level (6,9,8). Longer precursor transcripts with hairpin structures are first synthesized by RNA polymerase II, followed by processing of precursors by Drosha and Dicer. To date, miRNAs have been found to be involved in various physiological and pathological processes, including cell metabolism, tumorigenesis, cell growth and apoptosis, aging, organic development and the immune response (10,11). A single RNA may have hundreds or more targets, therefore it is difficult to distinguish functions to specific miRNAs.

Studies have demonstrated that miRNAs can function as oncogenes or tumor suppressors by regulating the expression of cancer-related genes (12). During the latest decade, a set of cancer regulator miRNAs have emerged and these are divided into oncomiRs or anti-oncomiRs. Specific miRNA profiles have been observed in both tumor tissues and plasma in various types of cancers (13-16). These miRNAs are believed to be potential biomarkers for the diagnosis and may also support the prognosis or treatment of cancer (17). Recent projects have attempted to decipher the differential expression of miRNAs in specific cancers; however, the complex pathways comprising miRNAs and their target genes remain unclear.

Identification of the molecular causes of cancer represents a major breakthrough in the history of medicine, moving the discipline from pattern recognition and therapeutic strategies based on molecular mechanisms and therapies derived from clinical trials (18). The development of pathway strategies for the analysis of gastric cancer makes it possible to use these approaches for future clinical treatment.

We previously reported that 5 significant miRNAs are overexpressed in gastric cancer and serve as a fingerprint for gastric cancer diagnosis (14). In the present study, the protein classes, molecular functions, biological functions and canonical pathways comprising the targets of each gastric cancer-related miRNA as well as four main canonical pathways were identified and analyzed, offering novel drug targets for gastric cancer therapy.

Materials and methods

Target prediction of gastric cancer (GC)-related miRNAs. A web-based software TargetScan (http://www.targetscan. org) was used to generate lists of possible gene targets of each miRNA (19). Then the targeted genes were inputted into another web server Panther (http://www.pantherdb.org/) which is designed for gene function cluster and we obtained the protein class from Panther analysis (20,21). After that, we clustered the same functional class of protein in top ten classes.

The web-based functional annotation tool Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (http://david.abcc.ncifcrf.gov/tools.jsp) has key components for disease analysis, gene ontology analysis and pathway analysis (22).

Signaling pathway mapping of GC-related miRNAs. The signaling pathways and processes were explored using the systems biology tool KEGG Mapper (http://www.genome.jp/ kegg/tool/map_pathway2.html) which is a collection of tools for KEGG mapping: KEGG pathway mapping, BRITE mapping and MODULE mapping (23). The KEGG database consists of 16 main databases (systems information, KEGG PATHWAY, KEGG BRITE, KEGG MODULE, KEGG DISEASE, KEGG DRUG and KEGG ENVIRON; genomic information, KEGG ORTHOLOGY, KEGG GENOME, KEGG GENES, KEGG SSDB and KEGG; chemical information, KEGG COMPOUND, KEGG GLYCAN, KEGG REACTION, KEGG RPAIR, KEGG RCLASS and KEGG ENZYME).

Results and Discussion

GC-related miRNAs. Based on the experimental data in our previous study, 5 miRNAs were selected as GC-related miRNAs (Table I). These miRNAs were found to be clearly increased in GC, and their dysregulation is believed to promote tumorigenesis.

There are 2 different precursors of miR-1, miR-1-1 and miR-1-2 (24). It exists alone or with miR-133a to form a cluster. It is well known that miR-1 is a tumor-suppressive miRNA in various types of cancers including lung cancer, gastric cancer, colorectal cancer, prostate cancer, thyroid cancer, head and neck squamous cell carcinoma and rhabdomyosarcoma (25-30), the effects of which have been confirmed to

Table I. The predicted targets of GC-related miRNAs.

GC miRNAs	No. of predicted target genes
miR-1	769
miR-20a	1,218
miR-27a	1,210
miR-34a	655
miR-423-5p	180

Table II. Molecular function and biological process analysis of GC-related miRNAs.

GC miRNAs	Molecular function and biological processes	Regulated by GC-miRNAs (%)
miR-1	Binding	34.40
	Catalytic activity	27.40
	Nucleic acid binding transcription	12.00
	Transporter activity	6.40
	Enzyme regulator activity	6.40
	Receptor activity	6.40
miR-20a	Binding	35.80
	Catalytic activity	28.60
	Nucleic acid binding transcription	13.10
	Enzyme regulator activity	6.80
	Receptor activity	5.30
miR-27a	Binding	32.40
	Catalytic activity	29.10
	Nucleic acid binding transcription	12.10
	Receptor activity	7.60
	Enzyme regulator activity	7.20
miR-34a	Binding	32.00
	Catalytic activity	25.80
	Nucleic acid binding transcription	11.40
	Enzyme regulator activity	8.70
	Receptor activity	8.70
miR-423-5p	Binding	34.50
	Catalytic activity	25.20
	Nucleic acid binding transcription	16.00
	Receptor activity	6.80
	Transporter activity	5.80

be mediated by oncogenes including MET proto-oncogene (MET), histone deacetylase 4 (HDAC4), forkhead box P1 (FOXP1), G1/S-specific cyclin D2 (CCND2), C-X-C chemokine receptor type 4 (CXCR4) and stromal cell-derived factor 1 (SDF-1) (25,28,31). Recent studies demonstrated similar tumor-suppressive function in hepatocellular
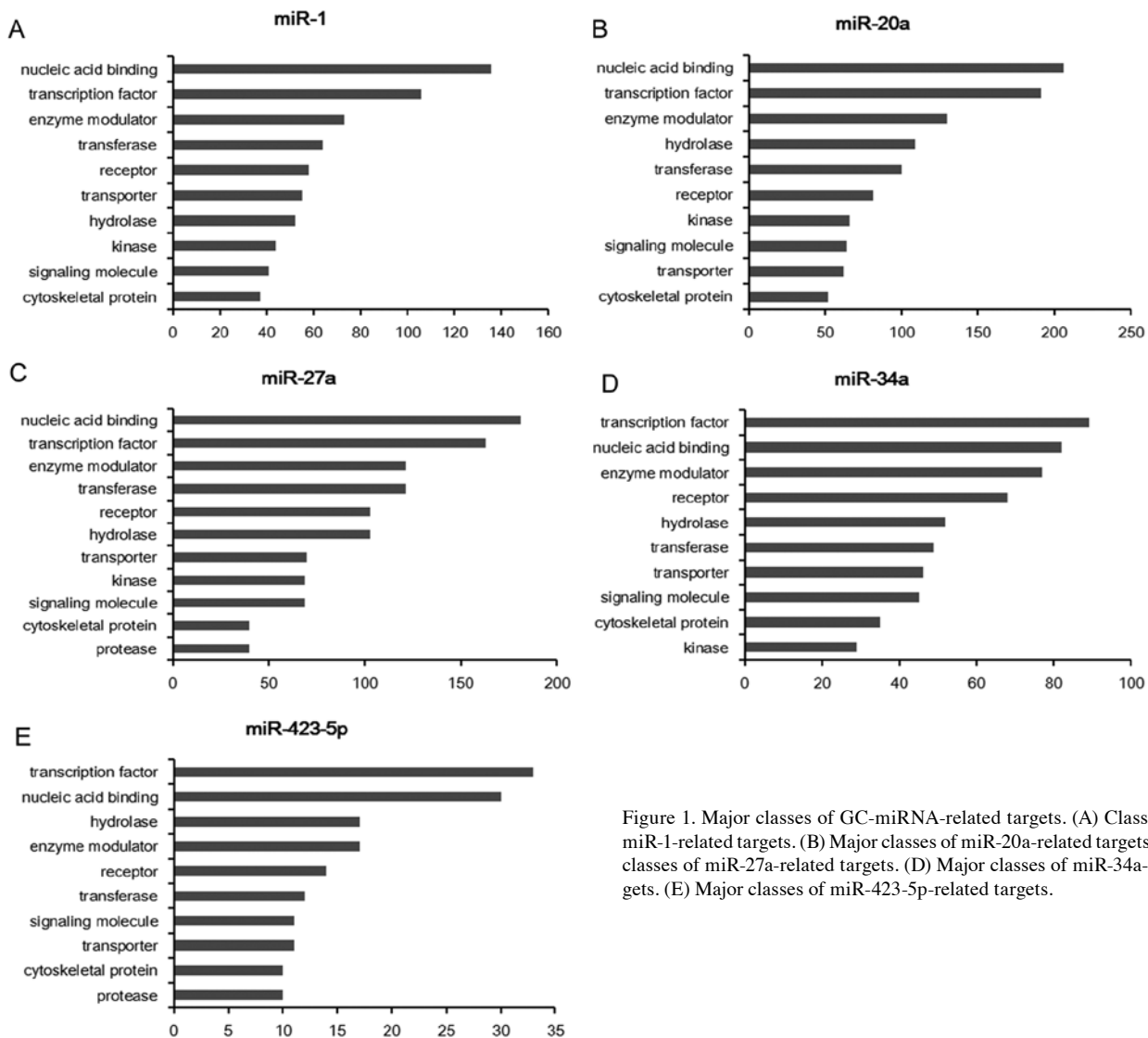
Figure 1. Major classes of GC-miRNA-related targets. (A) Classification of miR-1-related targets. (B) Major classes of miR-20a-related targets. (C) Major classes of miR-27a-related targets. (D) Major classes of miR-34a-related targets. (E) Major classes of miR-423-5p-related targets.

carcinoma (HCC) by targeting endothelin-1 (ET-1) and anti-apoptotic factor apoptosis inhibitor 5 (API-5) (32,33). Other studies revealed that re-expression of miR-1 may be a potential therapeutic target suppressing the malignant potential of lung cancer by mesenchymal-to-epithelial transition (MET) via downregulation of Slug, a member of the snail family of transcription factors (34). Similarly, restoration of the expression and the function of miR-1 leads to oncogene-driven reduction in cell proliferation, thus making miR-1 a promising therapeutic target for a multitude of cancers.

miR-20a belongs to the miR-17-92 cluster, also known as oncomiR-1, which includes 6 microRNAs: miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a-16 (35). It contributes to the regulation of many types of tumors as a tumor-suppressor in oral squamous cell cancer (36) and hepatic cancer (37) or a tumor-promotor in osteosarcoma (38), bladder cancer (39), GC (40), prostate cancer (41) and cervical cancer (42). It promotes the growth, migration, and invasion of GC cells by inhibiting the early growth response 2 (EGR2) signaling pathway (40), and may be related with the malignant process of cervical cancer, particularly invasion and metastasis by targeting autophagy-related protein 7 (ATG7), tissue

inhibitors of metalloproteinase 2 (TIMP2) and tankyrase 2 (TNKS2) (43). On the other hand, miR-20a is involved in the tumor inhibition of cutaneous squamous cell carcinoma (CSCC) by targeting LIM kinase-1 gene (LIK1), a metastasis promoter (44). Moreover, it targets MHC class I chain-related molecule A and B (MICA/B) to avoid NKG-mediated immune attack, thus enhancing the survival of ovarian cancer cells by immune escape (45). It has been shown that miR-20a inhibits the proliferation and metastasis of pancreatic carcinoma cells by directly downregulating Stat3 which is related to various physiological functions (46).

Previous studies have demonstrated that miR-27a acts as an oncogenic miRNA. Its role in promoting cell proliferation, invasion and metastasis has been verified in many malignancies, such as breast cancer (47), HCC (48), non-small cell lung cancer (NSCLC) (49), osteosarcoma (50) and renal cancer (51). In HCC, miR-27a promotes cell proliferation through suppression of its target gene peroxisome proliferator-activated receptor γ (PPAR-γ) (48). Direct and indirect mechanisms by which miR-27a can regulate both MET and EGFR, thus contributing to tumor progression, was discovered in NSCLC (49). Other studies emphasize

Table III. Pathway analysis of GC-related pathways.

GC miRNAs	Pathway	No. of genes (%)
miR-1	Gonadotropin releasing hormone receptor pathway	23 (6.00)
	Angiogenesis	20 (5.20)
	Wnt signaling pathway	18 (4.70)
	Integrin signaling pathway	13 (3.40)
	EGF receptor signaling pathway	13 (3.40)
	VEGF signaling pathway	12 (3.10)
	PDGF signaling pathway	12 (3.10)
	FGF signaling pathway	12 (3.10)
miR-20a	Wnt signaling pathway	39 (7.60)
	Gonadotropin releasing hormone receptor pathway	34 (6.60)
	Cadherin signaling pathway	25 (4.90)
	PDGF signaling pathway	23 (4.50)
	Angiogenesis	20 (3.90)
	Integrin signaling pathway	19 (3.70)
miR-27a	Gonadotropin releasing hormone receptor pathway	40 (6.40)
	Wnt signaling pathway	27 (4.30)
	Angiogenesis	23 (3.70)
	EGF receptor signaling pathway	22 (3.50)
	Inflammation mediated by chemokine and cytokine signaling pathway	20 (3.20)
	PDGF signaling pathway	18 (2.90)
	Integrin signaling pathway	18 (2.90)
	FGF signaling pathway	18 (2.90)
miR-34a	Gonadotropin releasing hormone receptor pathway	19 (5.80)
	Angiogenesis	14 (4.30)
	Inflammation mediated by chemokine and cytokine signaling pathway	11 (3.40)
	Wnt signaling pathway	10 (3.10)
	EGF receptor signaling pathway	10 (3.10)
	TGF-β signaling pathway	9 (2.80)
	Integrin signaling pathway	9 (2.80)
miR-423-5p	Inflammation mediated by chemokine and cytokine signaling pathway	5 (6.80)
	Wnt signaling pathway	5 (6.80)
	Gonadotropin releasing hormone receptor pathway	5 (6.80)
	Huntington disease	3 (4.10)
	Heterotrimeric G-protein signaling pathway-Gi α and Gs α mediated pathway	3 (4.10)
	PDGF signaling pathway	3 (4.10)

the role of miR-27a expression in sensitivity to anticancer therapies, including chemotherapy (52-58), radiotherapy (59) and thermal therapy (60). Downregulation of miR-27a is significantly associated with the expression of P-glycoprotein and multidrug resistance gene 1 (MDR1), leading to increased chemosensitivity through different targets, for example, FZD7/β-catenin pathway (52), homeodomain-interacting protein kinase 2 (HIPK2) (54) and RUNX1 (53). Notably, miR-27 can indirectly affect chemosensitivity by acting on the tumor microenvironment through transformation of normal fibroblasts to cancer-associated fibroblasts (55). Single nucleotide polymorphism rs11671784 is in the loop of pre-miR-27a and the G/A variation can significantly decrease the expression of mature miR-27a, followed by increased RUNX-1 expres-

sion and weakened chemosensitivity (53). CDC27 is a target of miR-27a by which radiosensitivity is modulated in triple-negative breast cancer (59). miR-27a may even contribute to thermal sensitivity by modulating HSP expression (60).

miR-34a is one of the earliest known tumor suppressors and is commonly downregulated in various solid cancers by targeting numerous oncogenes related to proliferation, apoptosis and invasion (61-68). miR-34a is downregulated in many cancers due to chromosomal deletion or CpG island methylation (69). As a direct transcriptional target of p53, decreased expression of miR-34a is partially due to mutations of p53 in several tumors (70). Ectopic expression of miR-34a can lead to cell cycle arrest, apoptosis or senescence, mimicking p53 activation (71). And miR-34a can suppress tumor metastasis
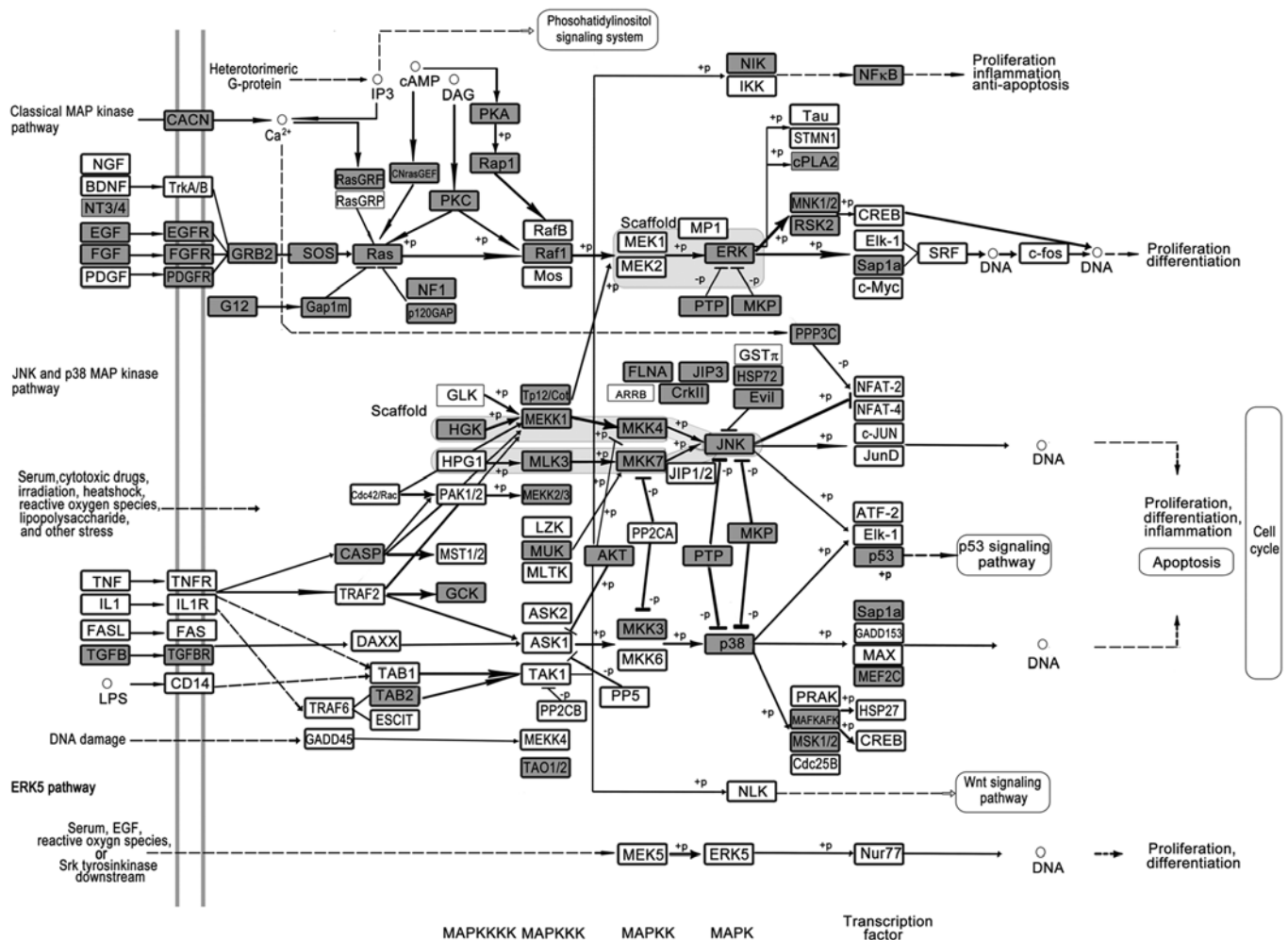
Figure 2. The MAPK signaling pathway is regulated by GC miRNAs. Grey boxes show objects that can be regulated by LC miRNAs. Solid arrows indicate activation; dashed arrows indicate indirect effect and ⊥ indicates inhibition. Letters on lines denote the type of regulation: -p, dephosphorylation; +u, ubiquitination.

and invasion through multiple targets in a variety of signaling pathways. For example, miR-34a targets FMNL2 and E2F5, then suppresses the progression of colorectal cancer (66). In prostate cancer, miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44 (65). It is verified that miR-34a inhibits gastric cancer proliferation and invasion via the downregulation of MET, meanwhile it is associated with the clinicopathological features of gastric carcinoma and can be a valuable predictor of patient prognosis (67). Epithelial-mesenchymal transition (EMT) is a key step in tumor progression. miR-34a inhibits EMT by targeting Smad4 through the transforming growth factor-β/Smad pathway in human cholangiocarcinoma (72). The role of miR-34a in regulating tumor biology has been extensively studied, while being an effective biomarker is another function of miR-34a. Serum miR-34a can be a potentially useful diagnostic biomarker of pancreatic ductal adenocarcinoma (73). Furthermore, miR-34a is likely to be involved in the treatment response of lung metastases of HCC to sorafenib (74).

The miR-423 family, identified as oncogenic miRNAs, has been explored in various types of cancers. There are two members of the miR-423 family, miR-423-3p and miR-423-5p. miR-423 plays a role in promoting cell growth and regulating G(1)/S transition by targeting p21$^{Cip1/Waf1}$ in HCC, however, miR-423-3p contributes to these effects, whereas miR-423-5p does not (75). Another study found that miR-423-5p is involved in autophagy regulation in HCC cells (76). Regarding the target of miR-423-5p, it has been demonstrated that it regulates cell proliferation and invasion by targeting trefoil factor 1 (TFF1) in GC cells (77). The expression level of miR-423-5p is aberrant in many cancers, including GC (14), colon carcinoma (78), pancreatic cancer (79) and breast cancer (80). A five-microRNA signature identified from genome-wide serum microRNA expression profiling, including miR-423-5p, can serve as a fingerprint for GC diagnosis (14). In stage I-II colorectal cancer, serum miR-423-5p was found to be significantly elevated compared with a healthy control, suggesting its value as a tool for early diagnosis (78). Furthermore, other than the diagnostic value of miR-423-5p, its role in prediction of the cancer therapeutic effect has been discovered. A classifier consisting of seven miRNAs, including overexpression of miR-423-5p, was able to identify a subgroup of glioblastoma patients who were resistant to temozolomide (81). On the contrary, the elevation of secretory miR-423-5p can be a favorable marker of the effect of sorafenib in HCC patients (76).
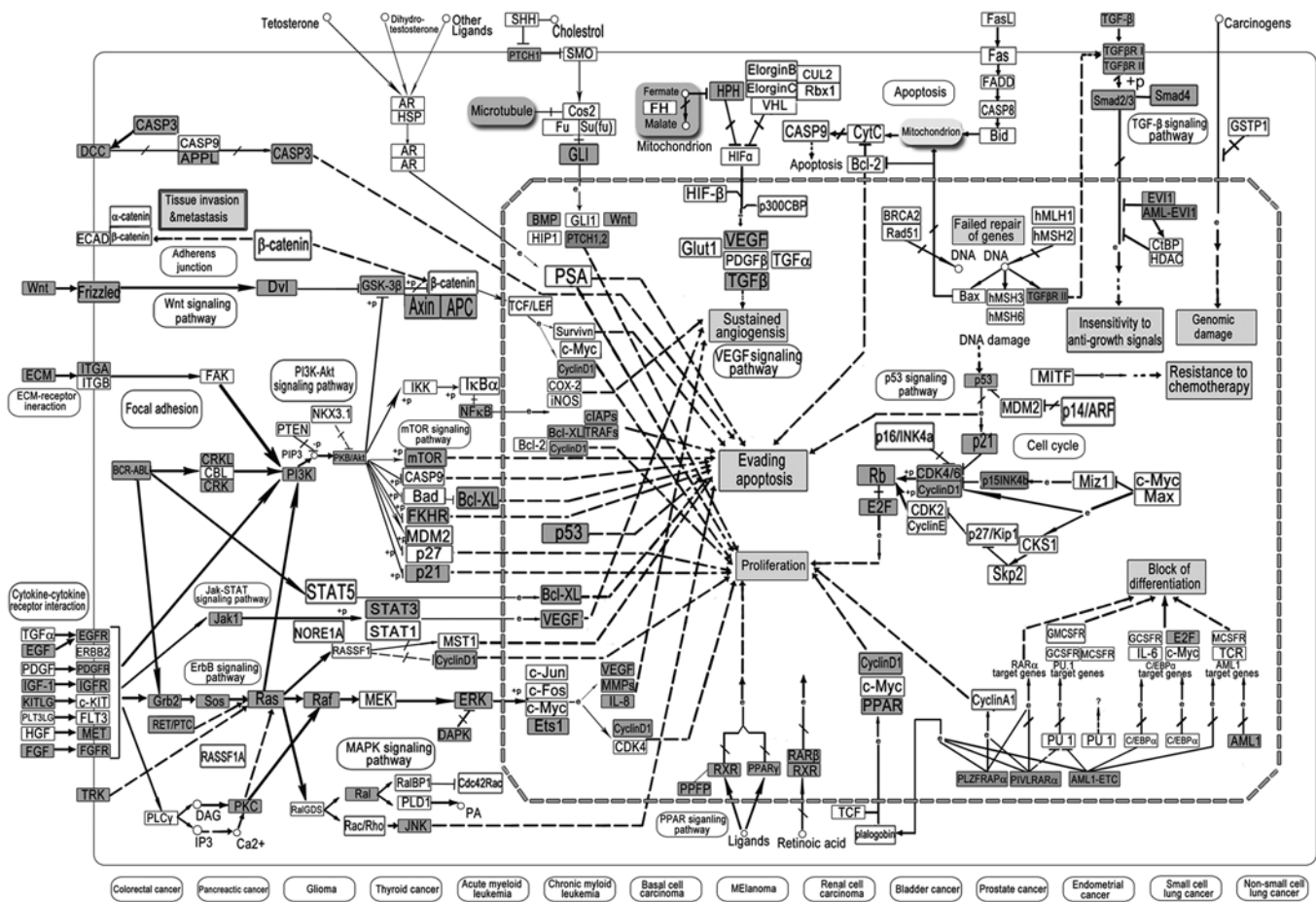
Figure 3. A schematic diagram of pathways in cancer regulated by GC miRNAs. Grey boxes show objects that can be regulated by LC miRNAs. Solid arrows indicate activation; dashed arrows indicate indirect effect and ⊥ indicates inhibition. Letters on lines denote the type of regulation: -p, dephosphorylation; +u, ubiquitination.

Predictions and protein classification of GC-miRNA targets. A single miRNA has hundreds of potential targets in physiological and pathological conditions; therefore, investigation of miRNA-target genes provides a better description of the miRNA-involved pathways. All of the predicted targets should be analyzed to fully understand the functions of GC-related miRNAs. As shown in Table I, GC miRNAs or miRNA family has the ability to directly target between 180 and 1,218 mRNAs of genes; moreover, a unique miRNA was observed to have multiple binding sites in the 3'UTR of the mRNAs. In total, 4,032 genes are regarded as downstream targets of the 5 significant GC miRNAs. Most of the genes are potential targets of oncomirs, and these genes are most likely to be downregulated in GC cells. Among all of the predicted genes, several important regulators include: BCL3, CD69, VIP, BMP3, MAPK1, BCL9L, BCL11B, PTEN; these genes are well known to be involved in cell apoptosis, cell proliferation, cell metastasis and angiogenesis.

As shown in Fig. 1, the top 1 class of targets for miR-1, miR-20a and miR-27a includes nucleic acid binding; while the top 1 class of targets for miR-34a and miR-423-5p includes transcription factor. A total of 136 miR-1-targeting genes, 206 miR-20a-targeting genes, 181 miR-27a-targeting genes belong to the protein class of nucleic acid binding. A total of 89 miR-34a-targeting genes and 33 miR-423-5p targeting

genes are in the class of transcription factor. Moreover, the class of enzyme modulator, transferase, receptor, kinase and transporter are found in the top 10 classes of all the 5 miRNA-related targets. miRNAs are likely to play a role as more refined regulators of gene expression, the minor percentage of the targets contain diverse proteins such as cytokines, transporters, hydrolases, transferases.

In agreement with our research, some groups have reported that miRNA-related oncogenes and tumor suppressors clearly show different patterns in function, expression, chromosome distribution, molecule size, free energy, targets and transcription factors (82-85).

Analysis of molecular functions, biological processes and signaling pathways for GC-miRNA-related targets. To provide a direct look at the pathways implicated in all targets of the 5 miRNAs, all the targets were used for further pathway analysis. As shown in Table II, the important molecular function and biological processes are almost identical to the potential targets of the 5 miRNAs. miR-1 contributes to the biological process of binding (34.4%), catalytic activity (27.4%), nucleic acid binding transcription (12%), transporter activity (6.4%), enzyme regulator activity (6.4%) and receptor activity (6.4%). miR-20a-related targets play a role in binding (35.8%), catalytic activity (28.6%), nucleic acid binding transcription
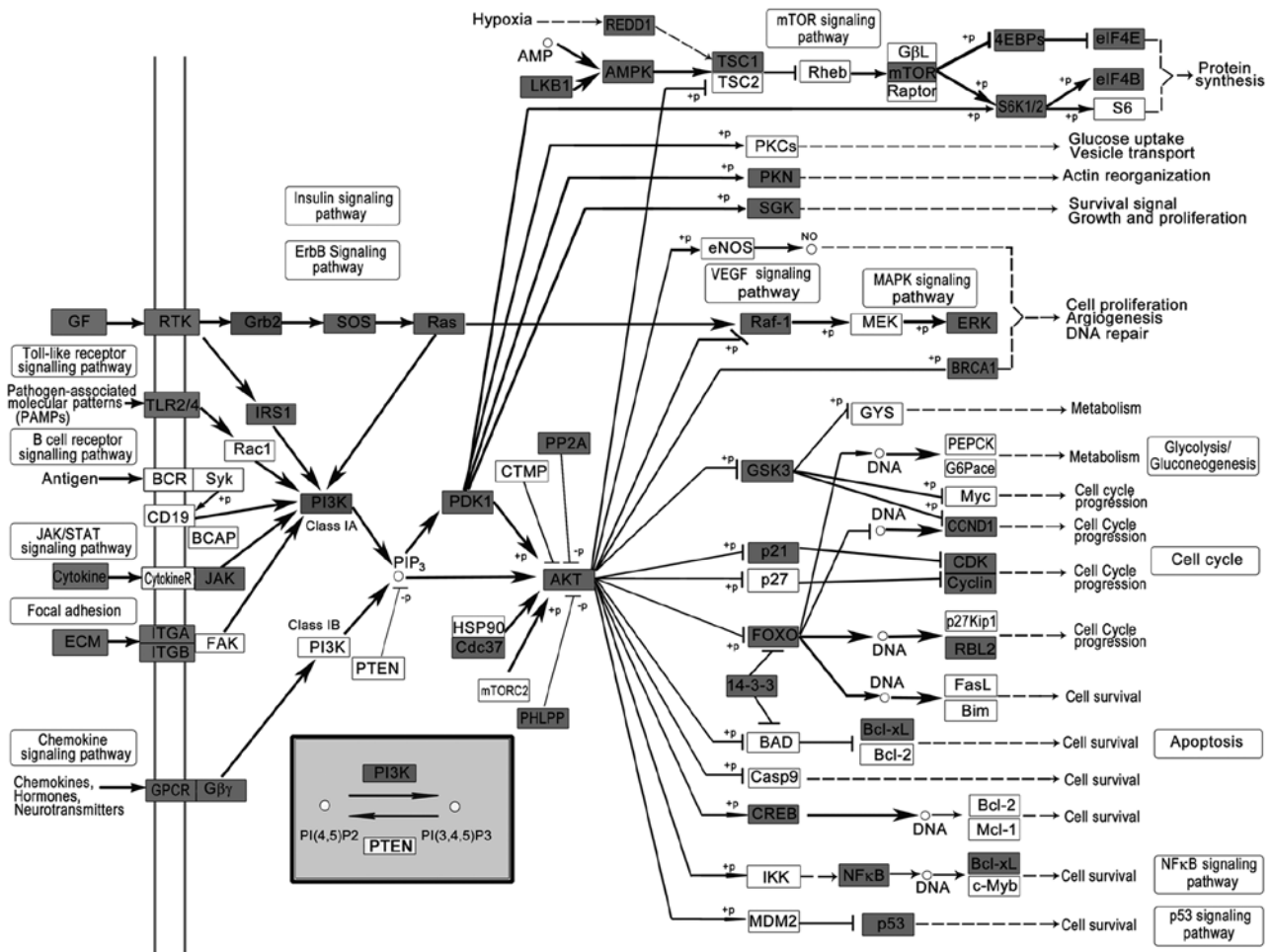
Figure 4. GC-miRNA regulation of the PI3K/Akt signaling pathway. Grey boxes show objects that can be regulated by LC miRNAs. Solid arrows indicate activation; dashed arrows indicate indirect effect and ⊥ indicates inhibition. Letters on lines denote the type of regulation: -p, dephosphorylation.

(13.1%), enzyme regulator activity (6.8%) and receptor activity (5.3%). The potential target genes of miR-27a are also involved in binding (32.4%), catalytic activity (29.1%), nucleic acid binding transcription (12.1%), receptor activity (7.6%) and enzyme regulator activity (7.2%). The miR-34a-related genes are expected to contribute to binding, catalytic activity, nucleic acid binding transcription, enzyme regulator activity and receptor activity. Furthermore, the target genes of miR-423-5p participate in binding (34.5%), catalytic activity (25.2%), nucleic acid binding transcription (16%), receptor activity (6.8%) and transporter activity (Table II).

The potential target genes of each miRNA were classified into several main groups, and the top 10 classes are respectively shown in Table III. miR-1, miR-27a, miR-34a and miR-423-5p are found to participate in the gonadotropin release hormone receptor pathway; miR-1, miR-20a, miR-27a and miR-34a are involved in the pathway of angiogenesis; miR-1, miR-27a and miR-423-5p are involved in Wnt signaling pathway (Table III). As predicted, miR-1 contributes to the pathways of EGF receptor signaling, VEGF signaling, PDGF signaling and FGF signaling, thus, the dysregulation of miR-1 is believed to play the most important role in the tumorigenesis in gastric cancer.

Pathway mapping of GC-miRNA-related targets. To give a direct view of the GC miRNA-related genes, all of the targets of miR-1, miR-20a, miR-27a, miR-34a and miR-423-5p were used for further pathway analysis. The 5 most important pathways include the MAPK signaling pathway (Fig. 2), PI3K-Akt signaling pathway (Fig. 3), pathways in cancer (Fig. 4), HTLV-I infection (Fig. 5) and the Ras signaling pathway (Fig. 6). These pathways are well known to play important roles in cell growth, cell metastasis, cell invasion and intercellular communication in various types of cancer (86-92).

As is shown is Fig. 2, the mitogen-activated protein kinase (MAPK) signaling pathway mainly affects the biological process of cell proliferation, differentiation, inflammation and the cell cycle. The MAPK signaling pathway is also associated with the p53 signaling and Wnt signaling pathways, two pathways that are known to determine tumorigenesis (Fig. 2). MAPK signaling is accurately regulated so that optimal biological activities are achieved and health is maintained; however, activation of the MAPK pathway is a frequent event in human cancer and is often the result of activating mutations in the BRAF and RAS oncogenes (93). Members of the MAP kinase family are evolutionarily conserved regulators that mediate signal transduction and play essential roles in various
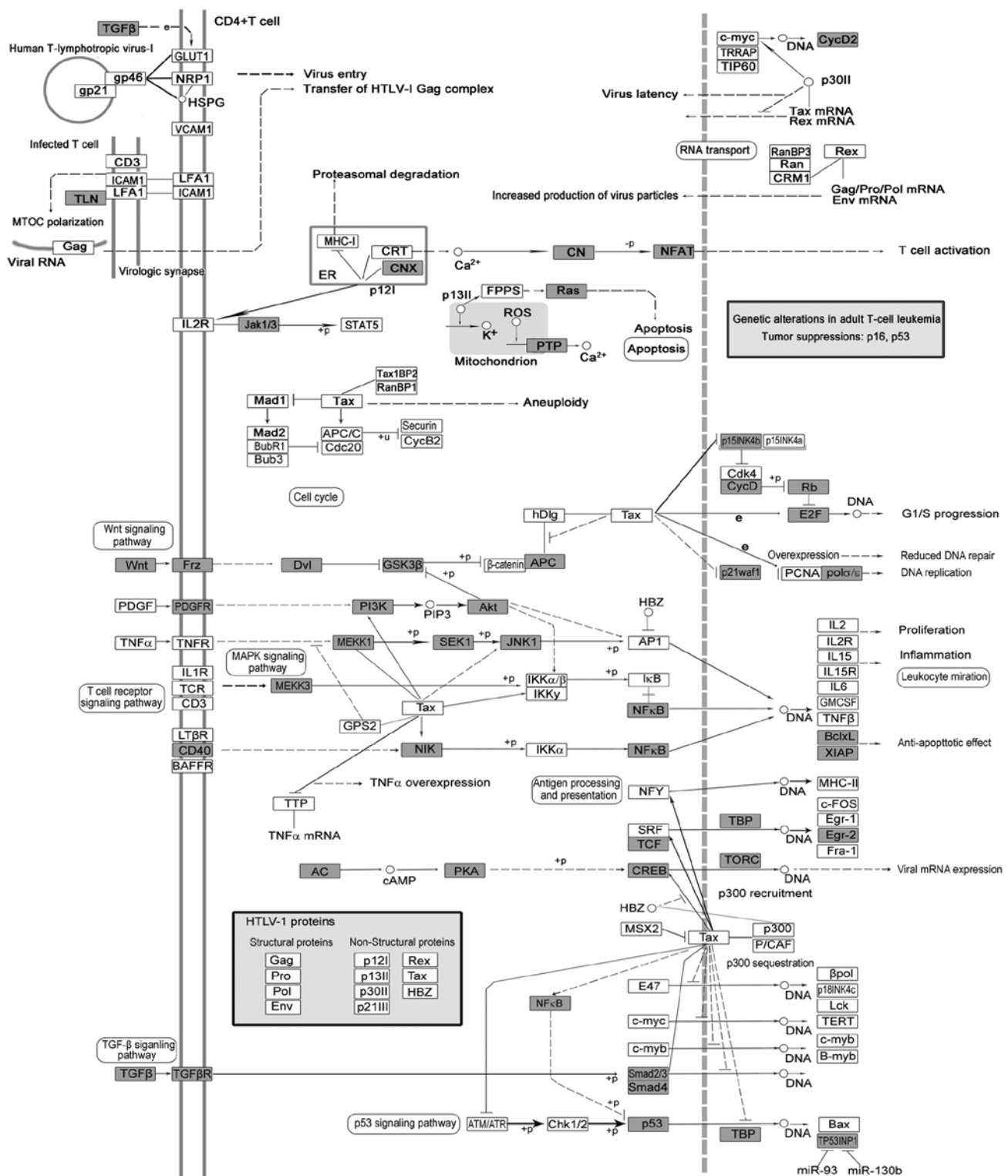
Figure 5. Regulation of the HTLV-1 pathway by GC miRNAs. Grey boxes show objects that can be regulated by LC miRNAs. Solid arrows indicate activation; dashed arrows indicate indirect effect and ⊥ indicates inhibition. Letters on lines denote the type of regulation: -p, dephosphorylation; +u, ubiquitination.

physiological processes. Previous studies in mouse models have demonstrated that MAPK controls cancer development, and these models are expected to provide novel strategies for cancer therapy (94). Although miR-155, miR-200 and miR-141 have been reported to regulate the expression of MAPK-related genes (95,96), the miRNA-MAPK pathway still needs further exploration.

Pathways in cancer are more closely linked with tumorigenesis, and include the MAPK, PI3K-Akt, VEGF, p53, PPAR and TGF-β signaling pathways (Fig. 3). As shown in Fig. 3, the pathways in cancer have demonstrated the acquisition of biological capabilities such as blockade of differentiation, resistance to apoptosis, unlimited replicative potential, sustained angiogenesis, tissue invasion and
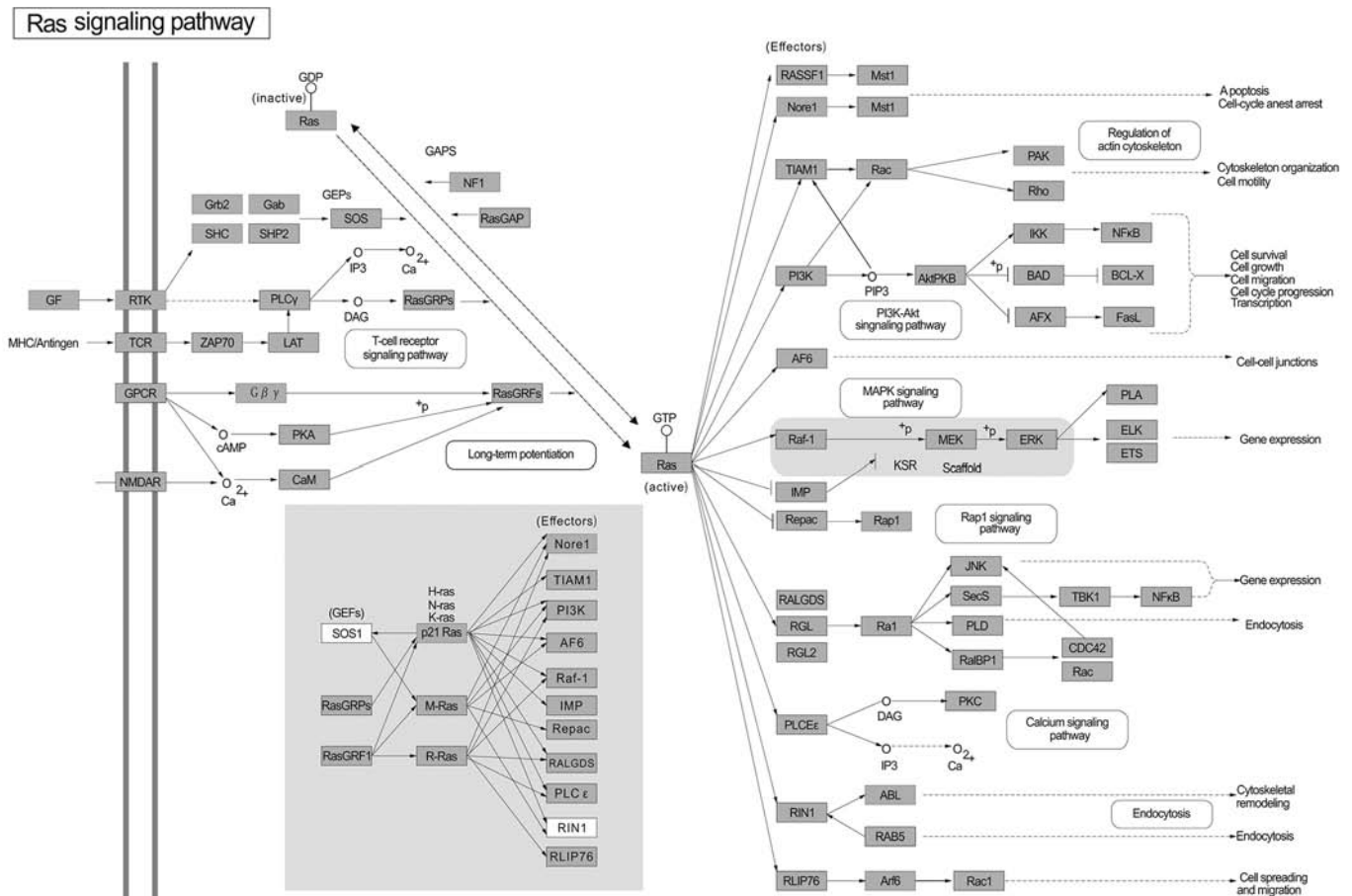
Figure 6. Analysis of the Ras signaling pathway regulated by GC miRNAs. Grey boxes show objects that can be regulated by LC miRNAs. Solid arrows indicate activation; dashed arrows indicate indirect effect and ⊥ indicates inhibition. Letters on lines denote the type of regulation: -p, dephosphorylation; +u, ubiquitination.

metastasis for the transformation of normal cells into highly malignant tumor cells. The important GC-miRNA-related genes are Wnt, STAT3, p21, p53, BCL-2, FAS and TGF-β; however, more common abnormalities in oncogenes and tumor-suppressor genes regulated by GC miRNAs can be used as potential therapeutic targets (Fig. 3).

The phosphatidylinositol-3-kinase (PI3K)-Akt signaling pathway (Fig. 4) has been reported to show frequent change in human cancer, and is thought to specifically interact with EGFR/ERBB family receptors (97-99). It is reported that the PI3K-Akt signaling pathway mediates regulation of p27, and is associated with cell cycle arrest and apoptosis in cervical cancer (100). In addition, the PI3K/Akt/mTOR pathway is also regarded as a therapeutic target in ovarian cancer (101). In bone cancer, cancer pain is closely linked with MCP-1 which stimulates spinal microglia, mediated by the PI3K/Akt pathway (102). In human prostate cancer, CD147 is reported to modulate autophagy though the PI3K/Akt/mTOR pathway in PC-3 cells (103). More inhibitors are designed to target the PI3K/Akt/mTOR pathway in clinical trials (104-107). In summary, numerous members of the PI3K/Akt pathway are crucial to many aspects of cell growth and survival, and are altered by amplification, mutation and translocation frequently, with resultant activation of the pathway.

Human T cell leukemia virus 1 (HTLV-1) is a retrovirus that causes adult T cell leukemia (ATL) and neurological disorder, the tropical spastic paraparesis (HAM/TSP) (Fig. 5). The pathogenesis apparently results from the pleiotropic function of Tax protein, which is a key regulator of viral replication (Fig. 5). Tax encoded by HTLV-1 has been implicated in tumorigenesis, and is involved in the dysregulation of anti-apoptosis and cell proliferation (108). Recently, miR-28-3p has been reported to be a cellular restriction factor that inhibits HTLV-1 replication and infection (109). However, the role of miRNA and HTLV-1 in cancer, particularly in GC, remains unclear.

Ras is another well-known regulator that activates many signaling cascades (Fig. 6); Ras genes encode proteins that are activated in an intracellular signaling network controlling differentiation, proliferation and cell survival (110-112). Ras mutations are common in human malignancies, especially in cancer, and have been identified in >30% of human cancers. Ras and the downstream proteins, Raf and MEK, play an important role in the development of malignancies, and often show frequent expression in cancers. Therefore, a variety of agents are designed to disrupt signaling though Ras or the downstream proteins (113).

In the present study, we constructed a detailed biological frame by in-depth analysis of the complex network comprising

GC-miRNA-related targets. These results are believed to provide a better understanding of the miRNA-regulated pathways in gastric cancer and identify novel potential targets for future clinical use.

Acknowledgements

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