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RESEARCH PAPER

IGF1/IGF1R and microRNA let-7e down-regulate each other and modulate proliferation and migration of colorectal cancer cells

Zhenjun Li[a], Weihuo Pan[a], Yi Shen[a], Zhiliang Chen[a], Lihua Zhang[a], Yuping Zhang[a], Quan Luo[b], and Xiaojiang Ying[a]

[a]Department of Colorectal Surgery, Shaoxing People's Hospital, Shaoxing, China; [b]Digestive Endoscopy Center, Shaoxing People's Hospital, Shaoxing, China

ABSTRACT

MicroRNA let-7 has been reported to be down-regulated in several human cancers and is now characterized as a tumor suppressor. IGF1R is over-expressed in many cancers and IGF1/IGF1R pathway is attractive target for anticancer therapy. However, the crosstalk between let-7 and IGF1/IGF1R are largely unknown. The present study showed IGF1R were significantly over-expressed in colorectal cancer tissues compared with adjacent normal tissues through immunohistochemical analysis. qRT-PCR results showed that let-7a, let-7b and let-7e were down-regulated in colorectal cancer tissues. Bioinformatics analysis revealed that both IGF1 and IGF1R mRNA are potential targets for let-7 miRNA family. Ectopic transfection of let-7e led to a significant reduction in IGF1R at protein level and their downstream Akt inhibition, as well as a reduction in cell proliferation, migration and invasion in colorectal cancer cells, while inhibition of let-7e enhanced the expression of IGF1R. On the other hand, IGF1 stimulation can significantly down-regulate the expression of let-7e in colorectal cancer cells. Taken together, our findings identify a negative feedback regulation between let-7e and IGF1/IGF1R, and suggest that let-7e could be used in IGF1R-targeted therapeutics in anticancer therapy.

Abbreviations: IGF1: insulin-like growth factor 1; **IGF1R**: IGF1 receptor; **miRNA**: microRNA; **CRC**: colorectal cancer; **EGFR**: epidermal growth factor receptor; **HRP**: horseradish peroxidase; **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; **p-Akt**: phospho-Akt; **PI3K**: phosphoinositide 3-kinase; **qRT–PCR**: quantitative reverse transcription–PCR; **IHC**: immunohistochemical; **siRNA**: small interfering RNA; **3'-UTR**: 3'-untranslated region

Introduction

Colorectal cancer (CRC) is among the five most common cancers in the world in terms of incidence for both men and women.[1] According to the American Cancer Society, the 3 most prevalent cancers among males are prostate (43%), colorectal (9%), and melanoma of the skin (7%), and those among females are breast (41%), uterine corpus (8%), and colorectal (8%) in the United States. It is estimated that there are nearly 1.2 million men and women living in the United States with a previous diagnosis of colorectal cancer, and an additional 143,460 will be diagnosed in 2012.[2]

The type I insulin-like growth factor receptor (IGF1R) is a member of a family of transmembrane tyrosine kinases. Accumulating evidence implicated the IGF-1R and its ligands, IGF-1 and IGF-2, in the development and progression of cancer including colorectal cancer.[3,4] The principal pathways for transduction of the IGF signal are the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathways.[5] Amplified IGF-1/IGF-1R signaling is not only associated with an increased relative risk for development of CRC, but also contributes to CRC cell survival, invasion, metastasis and resistance to chemotherapeutic drugs.[6,7] Although up-regulation of IGF1R has been found in many types of cancers including CRC, the mechanisms of the increase remains elusive.

Post-transcriptional gene regulation by miRNAs is an increasingly well-appreciated regulatory mechanism involved in almost every biological process of multicellular life.

Identified as the first mammalian miRNAs to have functional and sequence conservation to a C. elegans counterpart, the mammalian let-7 family comprises twelve members expressed from eight distinct loci (let-7a-1,-2,-3; let-7b; let-7c; let-7d; let-7e; let-7f-1,-2; let-7g; let-7i; miR-98).[8,9] Numerous reports have shown that the expression levels of let-7 are frequently low and the chromosomal clusters of let-7 are often deleted in many cancers. Let-7 is also a very attractive potential therapeutic target for anticancer drug development.[10,11] Intranasal administration of let-7 has already been found effective in reducing tumor growth in a transgenic mouse model of lung cancer.[12] Similar restoration of let-7 was also shown to inhibit cell proliferation in breast, colon and hepatic cancers, lymphoma, and uterine leiomyoma.[13–15] So let-7 has been characterized as tumor suppressor in several human cancers. It targets a number of proto-oncogenes, including Ras, HMGA2, c-Myc, IL6, and Lin28 and Lin28B.[8,16,17] Lin28/Lin28B function to block the post-transcriptional processing of let-7 to its mature, active cytoplasmic form by binding the primary and precursor forms of let-7. Hence, Lin28/Lin28B and let-7 are mutual antagonists; when expression and activity of one is high, the other is suppressed.[13–15,18]

Recent studies have pointed to a role of microRNAs (miRs) in post-transcriptional regulation of IGF1R.[19–25] While a group of miRs including miR-470, miR-669b and miR-681 are involved in repression of IGF1R in long-lived mutant mice [19], miR-375 and miR-7 target IGF1R in oesophageal and tongue squamous cell carcinoma cells, respectively.[20,21] miR-145 targets the docking protein of IGF1R, insulin receptor substrate-1, thus inhibiting IGF1R signaling.[22] In addition, miR-497 and miR-139 have been reported to target IGF1R and has a tumor suppressive role in human colorectal cancer.[24,25]

In the present study, through immunohistochemistry (IHC) assay, we found IGF1R were significantly over-expressed in CRC tissues compared with adjacent normal tissues. Using the online miRNA target prediction tool, we found let-7 has the highest probability score among the predicted miRNA targeting IGF1R (http://www.targetscan.org). There were three potential let-7-target sites with higher probability of preferential conservation among the 3′UTR of IGF1R. Moreover, IGF1 was also a potential target of let-7 according to the analysis of TargetScan. Through real-time RT-RCR, let-7a, let-7b, and let-7e were shown to be significantly down-regulated in CRC tissues compared with adjacent normal tissues. Furthermore, our data also showed that IGF1 stimulation can down-regulate the expression of let-7b and let-7e in colorectal cancer cell HCT-116. Over-expression of let-7e can significantly down-regulate the expression of IGF1R, and attenuate the proliferation, migration and invasion of HCT-116 cells.

Materials and methods

Tissue samples

All human tissue samples were obtained from patients undergoing surgical resection in Shaoxing People's Hospital. Informed consent was obtained from each patient before tissue collection. The tumor and its adjacent normal tissues were also obtained. Each specimen was divided into 2 parts. One of them was fixed in 10% phosphate-buffered formalin, embedded in paraffin and subjected to Immunohistochemical (IHC) analysis, and the other was used for extraction of miRNA.

IHC analysis

Sections were stained with Envision System method (DakoCytomation, Carpinteria, CA), according to manufacturer's instructions. Mouse monoclonal antibody against human IGFR1 was from Abcam. Saturation and intensity of immunostained cells was evaluated over 8 visual fields under a light microscope (Olympus Optical, Tokyo, Japan). In statistical analysis, with reference to previous study [26], total staining of IGF1R were scored as the product of the staining intensity (on a scale of 0–3: negative = 0, weak = 1, moderate = 2, strong = 3) × the percentage of cells stained (0 = zero, 1 = 1–25%, 2 = 26–50%, 3 = 51–100%), which resulted in a scale of 0–9. two independent investigators scored each sample without any prior knowledge of each patient's clinical information and outcome.

Cell culture, IGF1 treatment and transfection

The human colorectal cancer cell line HCT-116, LoVo, SW480 and SW620 were obtained from the American Type Culture Collection (ATCC) and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 U/mL streptomycin in a 5% CO_2 incubator at 37 °C. The normal human fetal colon cells (CRL-1831) were from ATCC and cultured in a 1:1 mixture of Ham's F12 and DMEM containing HEPES (25 mM), cholera toxin (10 ng/ml), insulin (5 μg/ml), transferring (5 μg/ml) and cortisone (100 ng/ml), and supplemented with 10% FBS. The cultures were maintained at 37°C in 5% CO_2.

For IGF1 treatment, when cells were grown to 30% confluency, and then medium was replaced with fresh serum-free medium containing 50 ng/ml IGF1 (PeproTech), and PBS were set as vehicle group. Cells were collected at 6 h, 12 h, and 24h after treatment for miRNA analysis.

Let-7e mimics and control mimics, let-7e antagomir and control antagomir were from Ribobio (Guangzhou, China). IGF1R-specific siRNA (On-TargetPlus SMARTpool) were from Dharmacon. miRNA transfection was performed using DharmaFECT (Thermo Scientific) reagent according to manufacturer's instructions. siRNA transfection was carried out with X-tremeGENE siRNA reagent from Roche according to manufacturer's instructions.

Western blot analysis

Antibody against IGF1R (#3027), pan Akt (#4691), p-Akt (Thr308, #4056) and p-Akt (Ser473, #4060) were purchased from Cell Signaling Technology. β-Actin (beyotime, #AF0003) was used as an internal control. In brief, after washing with ice-cold PBS three times, cells were homogenized and sonicated in RIPA buffer (50 mM Tris-base, 150 mM NaCl, 1.0 mM EDTA, 0.1% SDS, 1% Triton X-100, and 1% sodium deoxycholate) containing protease inhibitor cocktail (Sigma). Sample concentration was determined using the Bradford assay (Bio-Rad). Extracted proteins were separated by 12% SDS-PAGE and transferred to PVDF membranes. Membranes were blocked overnight with TBS containing 0.1% Tween 20 in 5% skimmed milk at 4°C, and then incubated with primary antibodies at room temperature for 1 h or overnight at 4°C. After three washes in TBST, the membranes were incubated with a HRP-conjugated secondary antibody for 2 h at room temperature, and the protein bands were then visualized using enhanced chemiluminescence reagents (Millipore).

miRNA extraction and real-time RT–PCR analysis

miRNA were extracted from freshly isolated clinical samples or from cultured cell lines by using miRcute miRNA Extraction Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. Reverse transcription was carried out using miRcute miRNA first-strand cDNA Synthesis Kit (TIANGEN). Realtime PCR was performed using miRcute miRNA qPCR Detection Kit (SYBR Green, TIANGEN) according to the manufacturer's instructions. U6

was used as a miRNA internal control. Let-7 family member-specific primer sets were as described previously.[27]

Proliferation and apoptosis assay

Cell proliferation was measured using the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. In brief, at 48 h post-transfection, the transfection medium in each well was replaced by fresh serum-free medium with MTT (1 mg/ml). After incubation at 37°C for 4 h, the medium were removed and 150 μl of dimethylsulfoxide were added to dissolve formazan crystals. Absorbance was measured at 570 nm using an ELISA microplate reader.

Cell apoptosis was determined by Flow cytometry analysis using Annexin V-FITC/PI apoptosis detection kit (beyotime) according to manufacturer's instructions. Briefly, at 48 h post-transfection, cells (1×10^5) were harvested and suspended in 195 μl binding buffer with 5 μl Annexin V-FITC, and incubated for 10 min at room temperature. After centrifugation, cells were re-suspended in 190 μl binding buffer with 10 μl propidium iodide. Samples were then analyzed by flow cytometry (Becton Dickinson).

Migration and invasion analysis

The ability of migration and invasion of colorectal cells were examined by using non-coated or Matrigel-coated transwell culture chamber (8 μm pore size, Corning) 48 h after transfection. For migration assays, 1×10^5 cells were seeded in the top non-coated chamber and incubated at 37°C for 8 h. For invasion assays, 5×10^4 cells were seeded in the top Matrigel-coated (BD Biosciences, Bedford, MA) chamber and incubated at 37°C for 24 h. In both assays, cells were suspended in serum-free RPMI 1640 medium in the upper chamber, and the lower chamber was filled with 1640 medium containing 5% FBS. After incubation, the top chambers were wiped with cotton wool to remove the non-migratory or non-invasive cells. Cells on the underside of the membrane were fixed in methanol for 30 min, stained with 0.1% crystal violet, and counted under a microscope (Eclipse TS100, Nikon, Japan).

Statistical analysis

All statistical analyzes were performed by Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

Results

Up-regulation of IGF1R in colorectal cancer

IHC analysis was performed to examine the expression of IGF1R in clinical colorectal cancer samples (Table 1 & Figure 1). As shown, IGF1R was weekly or moderately stained in the well-differentiated CRC tissues, whereas it was moderately or strongly stained in moderately- and poorly-differentiated CRC tissues. Of the 22 adjacent normal tissues, IGF1R was weekly or negatively stained. These result indicated that elevated expression of IGF1R was paralleled with increased severity of colorectal cancer (Figure 1(a)).

Table 1. IGF1R immunostaining results of patients with colorectal cancer.

Case	Male/Female	Cancer tissue type[a]	IGF1R Staining[b] Adjacent normal tissues	Cancer tissue
1	Female	Well	1 × 1	1 × 2
2	Female	Well	1 × 2	1 × 3
3	Female	Well	1 × 1	1 × 3
4	Male	Well	0 × 0	1 × 2
5	Male	Well	1 × 2	1 × 3
6	Male	Well	2 × 2	2 × 3
Average±SD (well) **			1.67 ± 1.37	3.17 ± 1.47[#]
7	Female	Moderately	1 × 2	2 × 3
8	Female	Moderately	0 × 0	1 × 3
9	Female	Moderately	1 × 1	2 × 2
10	Female	Moderately	1 × 2	2 × 3
11	Male	Moderately	2 × 2	3 × 3
12	Male	Moderately	1 × 1	1 × 3
13	Male	Moderately	0 × 0	2 × 3
14	Male	Moderately	1 × 1	2 × 3
15	Male	Moderately	2 × 2	1 × 3
16	Male	Moderately	0 × 0	2 × 3
Average±SD (Moderately) **			1.50 ± 1.51	5.20 ± 1.93[#]
17	Female	Poorly	1 × 2	3 × 3
18	Female	Poorly	1 × 1	1 × 3
19	Male	Poorly	2 × 2	3 × 3
20	Male	Poorly	1 × 2	3 × 3
21	Male	Poorly	1 × 1	3 × 3
22	Male	Poorly	1 × 2	2 × 3
Average±SD (Poorly) **			2.00 ± 1.10	7.50 ± 2.51[#]

a, Well, well-differentiated CRC; Moderately, moderately-differentiated CRC; Poorly, poorly-differentiated CRC.

b, IGF1R Staining was scored as the staining intensity (on a scale of 0–3: negative = 0, weak = 1, moderate = 2, strong = 3) × the percentage of cells stained (0 = zero, 1 = 1–25%, 2 = 26–50%, 3 = 51–100%).

**, $P < 0.01$ (between cancer tissue and pared adjacent normal tissues).

#, $P < 0.05$ (moderately-differentiated CRC and poorly-differentiated CRC compared with well-differentiated CRC separately).

IGF1R-targeted miRNA prediction and the down-regulation of let-7 miRNA in CRC

IGF1R-targeted candidate miRNA were predicted using using TargetScanHuman 6.2. Among the predicated candidate miRNA, let-7 miRNA family has the highest probability score (Figure 1(b)). As shown, there were three potential let-7-target sites with higher probability of preferential conservation among the 3′UTR of IGF1R. So we examined the expression of the nine let-7 members in CRC tissues through real-time RT-RCR. As shown in Figure 1(c), let-7a, let-7b, and let-7e were significantly down-regulated in CRC tissues compared with adjacent normal tissues. Among them, let-7e was the most significantly down-regulated member, and so it was selected for further research into the roles of let-7 dysregulation in CRC. Figure 1(d) showed that let-7e was down-regulated in four colorectal cancer cell lines (HCT-116, LoVo, SW480 and SW620) compared with the normal colon epithelial line CRL-1831.

Let-7e down-regulated the expression of IGF1R

As shown in Figure 2, transfection with let-7e mimic can reduce the protein levels of IGF1R in HCT-116 and LoVo colorectal cancer cells compared with control group. Conversely, knockdown of let-7e by let-7e-specific antagomir can cause an increase in the expression of IGF1R in HCT-116 and LoVo cells.
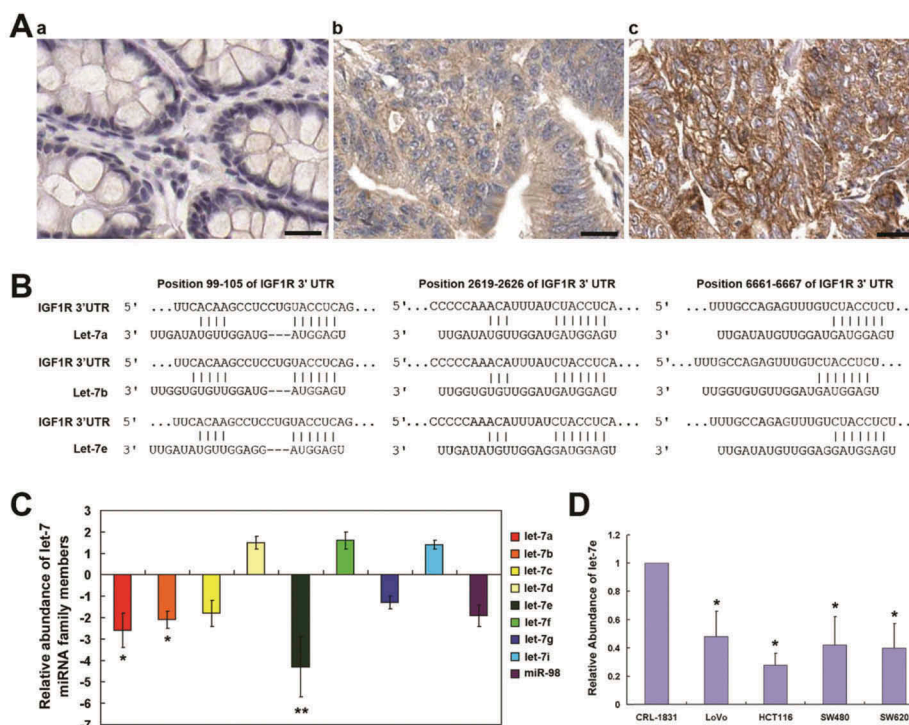
Figure 1. Expression of IGF1R in colorectal cancer, IGF1R-targeted miRNA prediction, as well as the expression of let-7 miRNA in CRC. (a) Immunohistochemical analysis of the expression of IGF1R in: a, adjacent normal tissues; b, moderately-differentiated CRC and c, pooly-differentiated CRC. (b) Let-7 miRNA family has the highest probability score among the predicted miRNA targeting IGF1R using TargetScanHuman 6.2. (c) qRT–PCR analysis of let-7 miRNA family in total RNA from CRC tissues and paired adjacent normal tissues. The data shown are average fold changes (the mean ± SD of three independent experiments) of individual miRNA expression CRC tissues relative to normal tissues. (d) qRT–PCR analysis of let-7 miRNA family in total RNA from indicated colon cancer cell lines and the normal colon epithelial line CRL-1831. The data shown are average fold changes (the mean ± SD of three independent experiments) of individual miRNA expression in each colon cancer line relative to CRL-1831 cells. *P < 0.05; **P < 0.01. Scale bar, 25 μm.
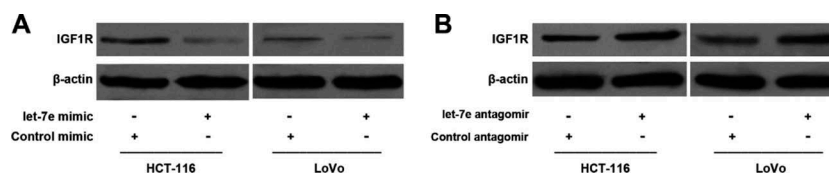

Figure 2. Effects of Let-7e on the expression of IGF1R. HCT-116 and LoVo human colorectal cancer cells were transfected with let-7e mimic or antagomir for 48 h. The expression levels of IGF1R were examined by Western blotting as described in the Methods section. Results shown are representative of at least three independent experiments.

Let-7e promoted apoptosis and inhibited proliferation, migration and invasion in CRC cells

The changes in cell proliferation, apoptosis, migration and invasion in CRC cells were examined by transfection of let-7e mimics. As shown in Figure 3(a), compared with control groups, let-7e mimics transfection can significantly inhibited the proliferation of both HCT-116 and LoVo cells using MTT assays. Though flow cytometry analysis using annexin V-FITC/PI double staining, it was shown that let-7e can promoted apoptosis both in HCT-116 and in LoVo cells. Moreover, transwell assays indicated the ability of migration and invasion in HCT-116 and LoVo cells were also significantly reduced by let-7e compared with control groups.

Let-7e inhibited the activation of Akt

Since IGF1R is a major player in the activation of the Akt signalling pathway and Akt activation play essential roles in

carcinogenesis, we investigated the effects of let-7e over-expression or IGF1R knockdown on Akt phosphorylation. As shown in Figure 4, although the total Akt level was not changed, the levels of p-Akt at both residues (Thr308 and Ser473) were reduced after transfection with let-7e mimic for 48 h both in HCT-116 cells and in LoVo cells and similar results were observed in IGF1R siRNA groups. These results suggest that let-7e and IGF1R play important roles in regulating Akt pathway in CRC cells.

Negative feedback regulation between microRNA let-7e and IGF1/IGF1R

Through bioinformatics analysis we found that let-7 miRNA family was also among the predicted miRNA targeting IGF1 using TargetScanHuman 6.2 (Figure 5(a)). We also investigated whether IGF1/IGF1R activation can influence the expression of let-7 miRNA in CRC cells. As shown in Figure 5(b), let-7a, let-7b and let-7e were significantly down-regulated following treatment
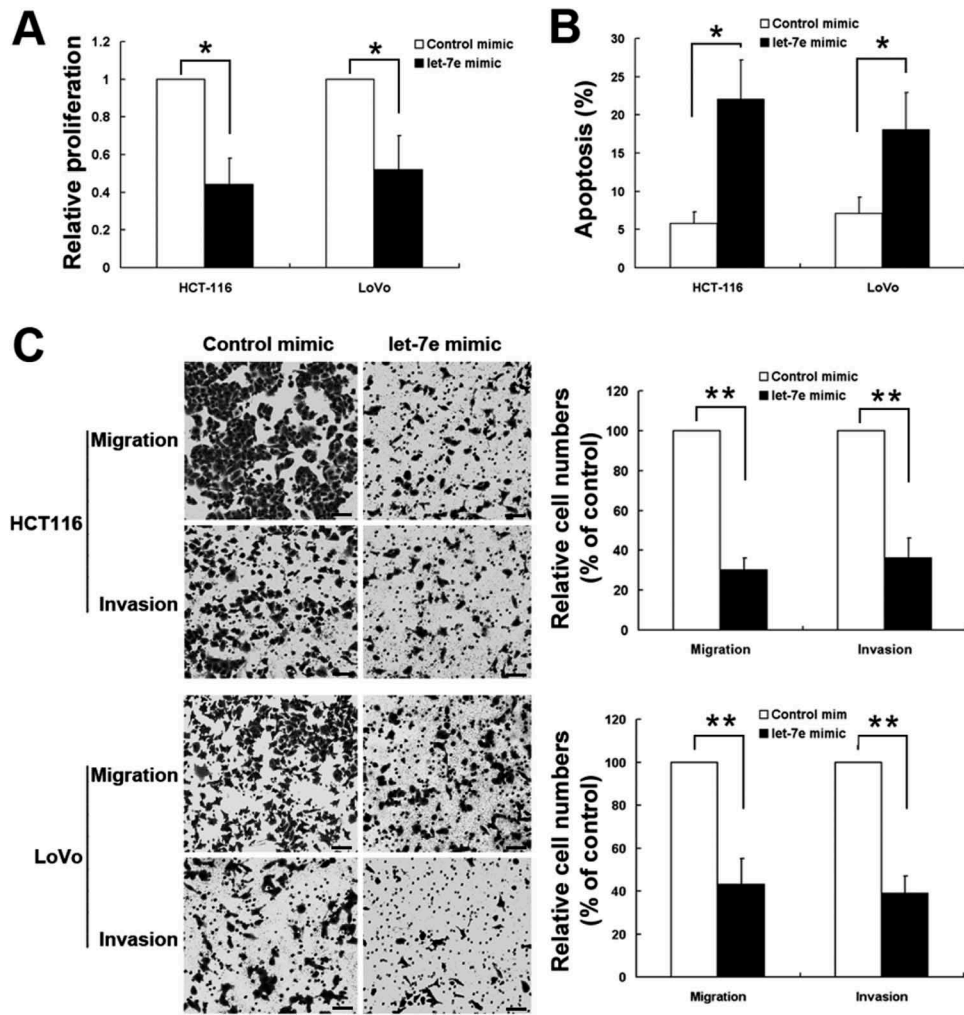
Figure 3. The effects of let-7e on cell proliferation, apoptosis, migration and cell invasion. After transfected with let-7e mimic, the changes in cell proliferation (**a**), cell-apoptosis rate (B), cell migration and invasion (c) were examined as described in the Methods section. All results shown are means ± SD from at least three independent experiments. *$P < 0.05$; **$P < 0.01$. Scale bar, 50 μm.
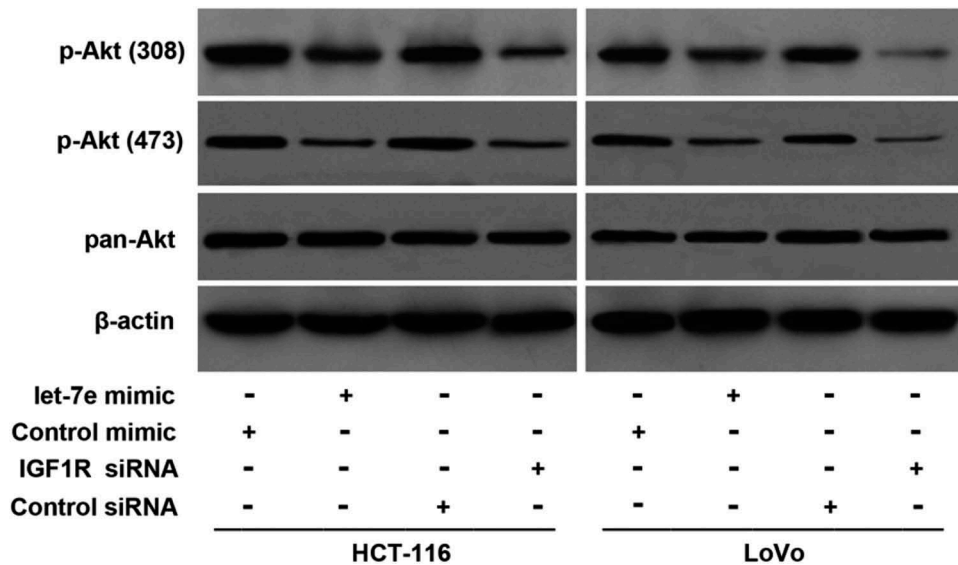

Figure 4. Effects of let-7e on activation of Akt. HCT-116 and LoVo human colorectal cancer cells were transfected with let-7e mimic or IGF1R siRNA for 48 h. The levels of total Akt (pan-Akt) and p-Akt (Thr308 and Ser473) were examined by Western blotting as described in the Methods section. Results shown are representative of at least three independent experiments.
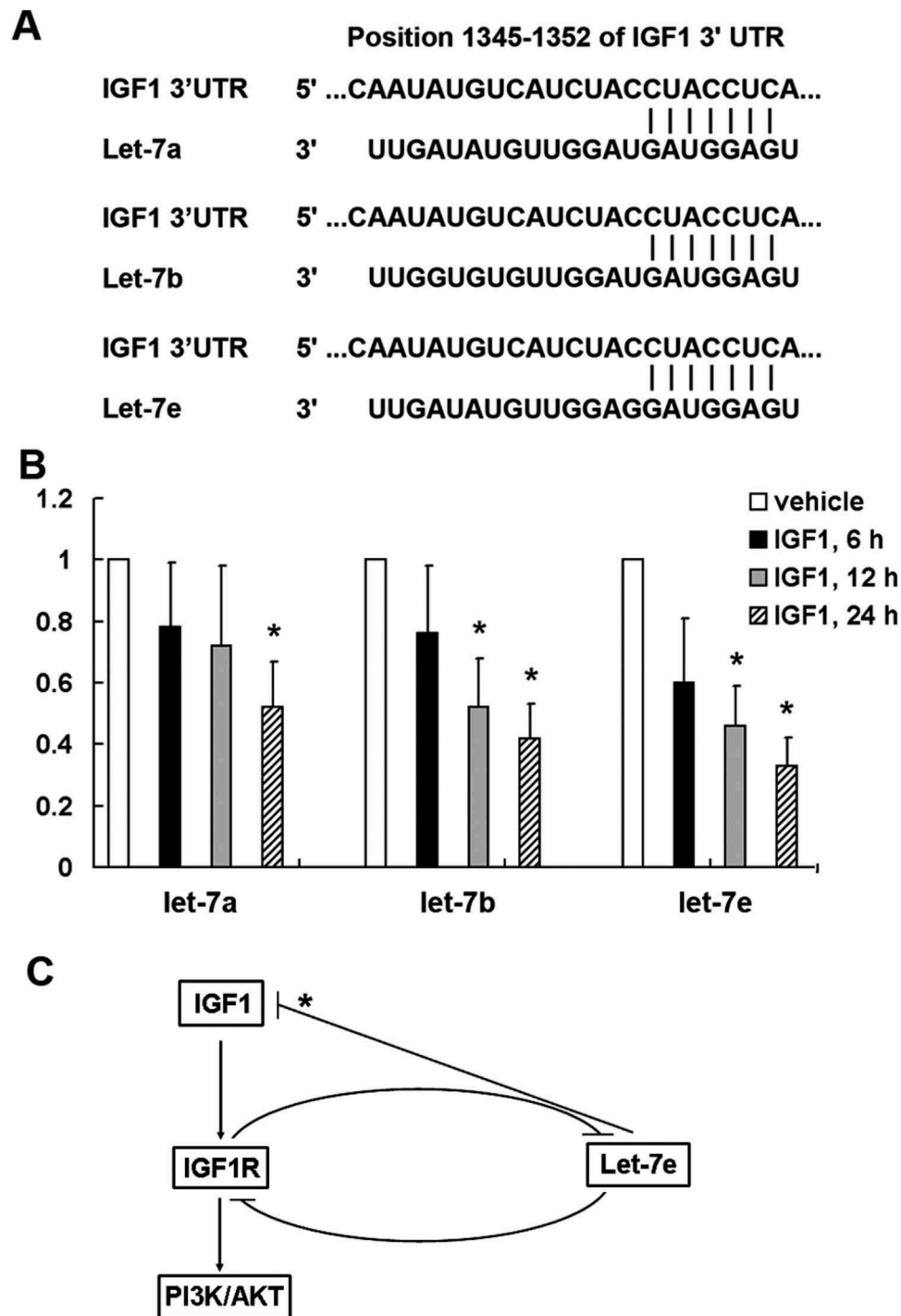
A

Position 1345-1352 of IGF1 3' UTR

IGF1 3'UTR   5' ...CAAUAUGUCAUCUACCUACCUCA...
                                 | | | | | | |
Let-7a   3'   UUGAUAUGUUGGAUGAUGGAGU

IGF1 3'UTR   5' ...CAAUAUGUCAUCUACCUACCUCA...
                                   | | | | | | |
Let-7b   3'   UUGGUGUGUUGGAUGAUGGAGU

IGF1 3'UTR   5' ...CAAUAUGUCAUCUACCUACCUCA...
                                   | | | | | | |
Let-7e   3'   UUGAUAUGUUGGAGGAUGGAGU

B

C

Figure 5. Negative feedback regulation between microRNA let-7e and IGF1/IGF1R. (a) Let-7 miRNA family is among the predicted miRNA targeting IGF1 using TargetScanHuman 6.2. (b) qRT–PCR analysis of let-7a, let-7b and let-7e in total RNA from HCT-116 colorectal cancer cell line after stimulated with IGF1 (50 ng/ml) for the indicated time. The data shown are average fold changes (the mean ± SD of three independent experiments) of individual miRNA expression relative to vehicle-treated cells. *$P < 0.05$. (c) Schematic diagram showing that activation of IGF1/IGF1R pathway can inhibit let-7e expression, and on the other hand over-expression of let-7e can inhibit the expression of IGF1 and IGF1R. * indicates a let-7e target predicted by TargetScanHuman 6.2 tool, but not validated experimentally in the present study.

with IGF1 in HCT-116 colorectal cancer cells by qRT–PCR analysis. Finally, schematic diagram showed a negative feedback regulation relationship between microRNA let-7e and IGF1/IGF1R (Figure 5(c)).

Discussion

The type 1 IGF receptor (IGF1R) is a transmembrane tyrosine kinase that is frequently overexpressed in human cancers. IGF1 binding to IGF1R stimulates downstream two main

proliferating pathways: the PI3K-Akt pathway and the Ras-MAPK pathway.[3,4] The result of MAPK pathway activation is increased cellular proliferation, whereas activating the PI3K pathway can inhibit apoptosis and stimulate protein synthesis, and thus IGF1R is crucial for tumor transformation and survival of malignant cell. IGF1/IGF1R signaling also plays important roles in hypoxia signalling, vasculogenesis, protease secretion, tumor cell motility and adhesion, and thus can affect cell propensity for invasion and metastasis.[3–5]

Emerging evidence suggests that IGF1/IGF1R pathway play a central role in the development and progression of CRC. [28–31] Oshima T et al. reported that IGF1R expression was correlated with venous invasion and liver metastasis, and thus was a useful predictor of liver metastasis from colorectal cancer.[28] Reinmuth N et al. suggested that the IGFIR plays an important role in multiple mechanisms that mediate the growth, angiogenesis, and metastasis of human colon cancer, and thus is a valid target for the therapy of human colon cancer.[29] It was also reported that amplified IGF-1/IGF1R signaling is not only associated with an increased relative risk for development of CRC, but also contributes to CRC cell survival, invasion, metastasis and resistance to chemotherapeutic drugs.[6,7] In the present study, through IHC analysis, we found that IGF1R was weekly or moderately stained in the well-differentiated CRC tissues, whereas it was moderately or strongly stained in moderately- and poorly-differentiated CRC tissues, indicating elevated expression of IGF1R was paralleled with increased severity of colorectal cancer. Colorectal carcinomas have 10-50 times higher levels of IGF-I and – II compared with adjacent normal colonic mucosa.[30–32] And it is known that the source of the most IGFI is the liver, the most frequent site of metastasis from colorectal cancer. Therefore, IGF1R inhibition may be an effective therapeutic approach against CRC.

MicroRNAs have been shown to be involved in a wide range of biological processes including cell differentiation, proliferation, apoptosis, and motility. Many researchers try to find the key miRNA targeting important oncogenes such as Ras, c-Met, EGFR, VEGFR, c-Myc and so on. Recent studies have found out some miRNA functionally involved in regulation of IGF1R, such as miR-195, miR-7, miR-139, miR-145, miR-375, miR-470, miR-497, miR-669, and miR-681.[19–25] In the present study, using the online miRNA target prediction tool, we found that miRNA let-7, known tumor suppressor, has the highest probability score among the predicted miRNA targeting IGF1R. There were three potential let-7-target sites with higher probability of preferential conservation among the 3′UTR of IGF1R. Moreover, IGF1 was also a potential target of let-7 according to the analysis of TargetScan.

Identified as the first mammalian miRNAs, let-7 family members have been widely reported that their expression levels are frequently low and the chromosomal clusters of let-7 are often deleted in many cancers. Let-7 is also a very attractive potential therapeutic target for anticancer drug development.[8–11] In the present study, through real-time RT-RCR, let-7a, let-7b, and let-7e were shown to be significantly down-regulated in CRC tissues compared with adjacent normal tissues. Moreover, ectopic transfection of let-7e led to a significant reduction in IGF1R at protein level and their downstream Akt inhibition, as well as a reduction in cell proliferation, migration and invasion in colorectal cancer cells, while inhibition of let-7e enhanced the expression of IGF1R. These results suggested that let-7e dysregulation may play an important role in the regulation of IGF1R expression and cell proliferation and metastasis in CRC.

Finally, through bioinformatics analysis we found that let-7 miRNA family was also among the predicted miRNA targeting IGF1. Conversely, let-7a, let-7b and let-7e were significantly down-regulated following treatment with IGF1 in HCT-116 colorectal cancer cells. So in the present study, we found a negative feedback regulation relationship between microRNA let-7e and IGF1/IGF1R.

In conclusion, the present study provides new insights into the role of IGF1/IGF1R and miRNA let-7e in colorectal cancer. Our data indicated that elevated expression of IGF1R was paralleled with increased severity of colorectal cancer. Let-7e was down-regulated in colorectal cancer. More importantly, ectopic transfection of let-7e led to a significant reduction in IGF1R at protein level and their downstream Akt inhibition, as well as a reduction in cell proliferation, migration and invasion in colorectal cancer cells. On the other hand, IGF1 stimulation can significantly down-regulate the expression of let-7e in colorectal cancer cells. The illustrated negative feedback regulation mechanisms can provide clues for new therapeutic strategies for colorectal cancer.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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