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MiR-150 alleviates EMT and cell invasion of colorectal cancer through targeting Gli1

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Abstract. – OBJECTIVE: Epithelial-mesenchymal transition (EMT) is related to colorectal cancer invasion and metastasis. Glioma-associated oncogene homolog 1 (Gli1) abnormal expression is associated with EMT, invasion, and metastasis in various cancers. MiR-150 is found downregulated in colorectal cancer pathogenesis. Bioinformatics analysis shows the complementary targeted relationship between miR-150 and the 3'-UTR of Gli1 mRNA. This study explores the role of miR-150 in regulating Gli1 expression, colorectal cancer cell EMT, and invasion.

MATERIALS AND METHODS: Dual l[illegible]ase assay confirmed the targeted relat[illegible] between miR-150 and Gli1 predicted by [illegible]n-formatics analysis. MiR-150 and Gli1 ex[illegible]sions were compared in NCM460, SW480, [illegible] SW620 cells. Cell colony formation and invasi[illegible] were tested in SW480 and SW[illegible] Anip97[illegible] and AGYZ83-a cells were t[illegible]d [illegible] ng/mL TGF-β1 to detect miR-150 [illegible] Gli1 ex[illegible]ssions. SW620 cells were cultu[illegible]n vitr[illegible] id-ed into five groups, in[illegible]lu[illegible]iR[illegible], [illegible] mimic, si-NC, si-Gli[illegible]d i[illegible] mimic + si-Gli1 groups.

RESULTS: MiR[illegible] specifically [illegible]ited Gli1 expression. T[illegible]e [illegible] of miR-150 [illegible] significantly down[illegible]lated, [illegible]e Gli1 was elevated in SW480 [illegible] SW620 cel[illegible]pared with that in NCM460 [illegible]s. SW620 exhi[illegible] markedly stronger in[illegible]e and colony forma[illegible]on abilities than SW4[illegible] he le[illegible] of miR-150 was apparently re-du[illegible] wher[illegible] Gli1 w[illegible]s increased in SW620 than [illegible]480 c[illegible] after the treatment of TGFβ1. [illegible]50 mi[illegible] and/or si-Gli1 transfec-[illegible]arke[illegible]d Gli1 and Snail levels, up-reg[illegible]ed E-c[illegible]n expression, and attenuat-ed [illegible] colony fo[illegible]nation and invasion.

CONCLUSIONS: Downregulation of miR-150 [illegible] of Gli1 promote the development [illegible]d invasion of colorectal cancer cell EMT. MiR-[illegible] attenuated the progression of colorectal [illegible] cell EMT via inhibiting Gli1.

Key Words:
miR-150, Gli1, EMT, Colorectal cancer, Invasion.

Introduction

Colorec[illegible] (CRC) is th[illegible]ost common malignan[illegible]umor [illegible]estive tract worldwide. It mainly occurs in [illegible]nction of the rectum and[illegible]d colon. Th[illegible]e range of 40-50 y[illegible] old represents the higher risk of CRC, the inciden[illegible]of male is 2-3 times higher [illegible] that of fe[illegible][1]. CRC is characterized as i[illegible]ve and m[illegible]static, which is related to poo[illegible]ct, high recurrence rate, and poor pr[illegible][illegible].

Epithelial-mesenchymal transition (EMT) re[illegible] the biological process of epithelial cell [illegible]g into mesenchymal cells. Downregulation of E-cadherin mediated tight junction between cells and extracellular matrix is an important indicator of EMT. Cancer cell EMT is closely related to tumor progression, metastasis, recurrence, and poor prognosis[3,4]. Hedgehog signaling pathway widely expresses in multiple tissues and cells, and it participates in regulating embryonic development and damage repair[5,6]. Physically, Hedgehog signaling pathway is in the resting status. It is involved in various tumor occurrence, development, metastasis, and recurrence when activated by gene mutation, overexpression, or other mechanisms[7-10].

Glioma-associated oncogene protein 1 (Gli1) is an important transcription factor in Hedgehog signaling pathway. It can regulate various intranuclear target genes transcription and expression, thus affecting cell proliferation, apoptosis, migration, and invasion. Gli1 overexpression can activate Hedgehog signaling pathway, which is associated with cancer occurrence, progression, and metastasis[11-13]. Gli1 was found abnormally increased in CRC tissue[14,15]. MiRNA is a type of endogenous single-stranded noncoding RNA with the length of 22-25 nt discovered in eukaryote. It plays a degradation

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or transcriptional inhibition role on mRNA via the binding between miRNA and 3'-UTR of mRNA, thus participating in cell proliferation, differentiation, and migration. MiRNA plays an oncogene[16] or tumor suppressor gene[17] in tumorigenesis. Recent researches[18,19] showed that miR-150 expression was reduced in CRC tissue. *In silico* study by bioinformatics analysis shows the complementary targeted relationship between miR-150 and the 3'-UTR of Gli1 mRNA. In our work, we aimed to investigate the effect and possible mechanism of miR-150 on colorectal cancer cell EMT

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Materials and Methods

Main Reagents and Materials

Human high metastatic CRC cell line SW620, low metastatic CRC cell line SW480, and normal colon epithelial cell line NCM460 were purchased from Jining Cell Culture Center (Shanghai, China). RPMI-1640, fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA, USA). EasyPure RNA Kit, Real-time PCR reagent TransScript Green One-Step qRT-PCR SuperMix were obtained from TransGen Biotech (Beijing, China). miR-NC, miR-150 mimic, miR-150 inhibitor, and riboFECT™ CP were bought from RiboBio (Guangzhou, China). Rabbit anti-human Snail and E-cadherin primary antibodies were obtained from CST (Cell Signaling Technology, Danvers, MA, USA). Mouse anti-Gli1 and β-actin primary antibodies were got from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Horseradish peroxidase (HRP) conjugated secondary antibody was derived from Bio-Rad (Hercules, CA, USA). Transwell chamber was bought from Merck Millipore (Frickenhausen, Germany). Matrigel was derived from BD Biosciences (Franklin Lakes, NJ, USA). Dual luciferase activity detection kit and pGL3 luciferase gene reporter plasmid were purchased from Promega (Madison, USA). TGF-β1 was obtained from Peprotech Biological (Beijing, China).

Cell Culture and EMT Induction

SW620, SW480, and NCM460 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium containing 10% FBS and 1% penicillin-streptomycin. The cells were passaged at 1:4.

For EMT induction, SW620 and SW480 cells were seeded in six-well plate at 3×10^4/well for 24 h. Then, the cells were treated by 10 ng/ml TGF-β1 for 48 h to induce EMT.

Dual-Luciferase Reporter Gene Assay

The PCR products containing full-length of Gli1 gene 3'-UTR segment from 293T cells were cloned to pGL3. Next, was transformed to DH5α competent cells and sequenced to select the plasmid with correct sequence. The pGL3-Gli1-3'-UTR-wt, pGL3-Gli1-3'-UTR-mut) was co-transfected 293T cells using riboFECT™ CP together with miR-150 mimic (or miR-NC). The luciferase activities were detected according to the Dual-Glo Luciferase Assay manual after cultured for 48 h.

Cell Grouping and Transfection

SW620 cells were cultured *in vitro* and divided into five groups including miR-NC, miR-150 mimic, si-NC, si-Gli1, and miR-150 mimic + si-Gli1 groups. Nucleotide fragments and riboFECT™ CP reagent were added to 1×riboFECT buffer and incubated at room temperature for 0-15 min, respectively. Then, they were added to the cells, which were used for detection after 72 h.

qRT-PCR

Total RNA was extracted using EasyPure RNA Kit and adopted for PCR reaction by TransScript Green One-Step qRT-PCR SuperMix. The reaction system contained 1 μg RNA template, 0.3 μM primers, 10 μL 2×TransStart Tip Green qPCR SuperMix, 0.4 μL RT Enzyme Mix, 0.4 μL Dye II, and ddH_2O. The PCR reaction was composed of 45°C reverse transcription for 5 min, 94°C pre-denaturation for 30 s, followed by 40 cycles of 94°C for 5 s and 60°C for 30 s. Real-time PCR was performed on Bio-Rad (Hercules, CA, USA) CFX96 connect to test the relative expression.

Western Blot

Total protein was extracted by SDS buffer from cells. A total of 40 μg protein was separated by 8-10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to membrane. Next, the membrane was blocked and incubated in primary antibody at 4°C overnight (E-cadherin, Gli1, Snail, and β-actin at 1:3000, 1:2000, 1:2000, and 1:10000, respectively). Then the membrane was incubated in secondary antibody (1:25000) for 60 min after

washed by PBST for three times. At last, the protein expression was detected by electrochemiluminescence (ECL).

Colony Formation Assay

The cells were seeded in 10 cm dish at 100/well and cultured at 37°C and 5% CO_2 for 14-21 days. The dish was washed by phosphate buffered saline (PBS) for twice and fixed by 4% paraformaldehyde when the macroscopic clone appeared. Next, the dish was stained by Giemsa for 20 min to count the clone with more than 10 cells. At last, the colony formation rate was calculated by (clone number/seeded cell number) ×100%.

Transwell Assay

A total of 500 μl RPMI-1640 medium containing 10% FBS were added to the 24-well plate. Then, the Transwell chamber paved 100 μl Matrigel was put onto the plate and added with SW620 cells resuspended in 200 μl serum-free RPMI-1640 medium (1×10⁶/mL). After 48 h, the membrane was fixed in methanol and stained by crystal violet. At last, the membrane was observed under the microscope.

Statistical Analysis

All data analyses were performed with S… 18.0 software (SPSS Inc., Chicago, IL, USA). … measurement data were depicted … an ± stan… dard deviation and compared … $p < 0.05$ … was considered as statistica… gnifica…

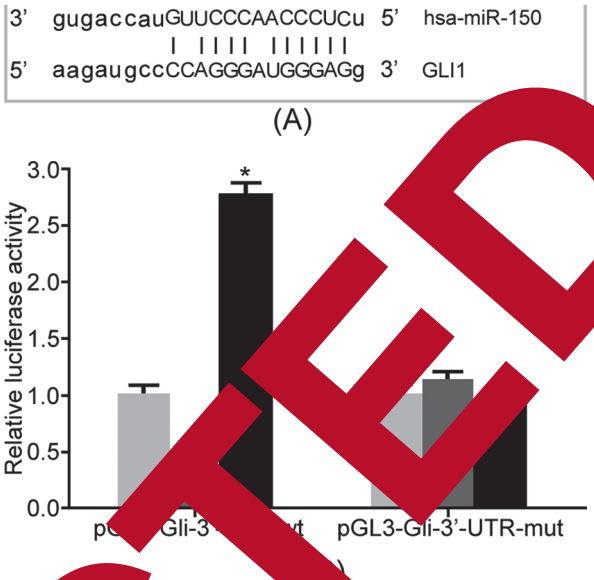
…ults

MiR-150 Inhi… …li1 Expres…

Online tar… gene … iction by microRNA.org showed … targeted b… site between miR-150 and … TR of Gli1 mR… igure 1A). Dual lucife… assay … evealed that miR-150 mimics or i… tor tr… ection significantly declined or eleva… …ve luci… se activity of HEK293 cells (1… B), in… ng the regulatory rela… ip be… n… 50 and Gli1 mRNA.

Mi… 50 was …ownregulated, while Gli1 W… …ressed in CRC Cells

…he detection of qRT-PCR showed that Gli1 …NA expression was highest in SW620 cells, …ed by that in SW480 and NCM460 cells (Fi… e 2A). Also, miR-150 level was lowest in SW620 cells, compared with that in SW480 and NCM460 cells (Figure 2B).



Fi… 1. MiR-150 targeted …gulated Gli1 expression. …he binding site b…tween miR-150 the 3'-UTR of Gli1 …A. ***B,*** Dual luc… se assay. *$p < 0.05$, compared with …IC.

Gli1 W… …reased, While miR-150 Was …Reduced During the EMT Process

…y formation assay demonstrated that …one… rmation capability in SW620 cells was markedly stronger than that in SW480 cells (Figure 3A, B). qRT-PCR showed that the level of miR-150 was declined, and Gli1 mRNA was markedly enhanced in SW620 and SW480 cells treated by TGF-β1, among which stronger metastatic ability was found in SW620 cells (Figure 3C, D).

The Overexpression of miR-150 Downregulated Gli1 Level, Suppressed EMT Process, and Attenuated Cell Invasion in CRC Cells

After the transfection of miR-150 mimic and/or si-Gli1, Gli1, and Snail levels were markedly reduced, E-cadherin expression was upregulated (Figure 4A), and cell colony formation (Figure 4B) and invasion were attenuated (Figure 4C).

Discussion

Hedgehog (Hh) gene is originally found in drosophila genetic mutation. Hedgehog signaling pathway is activated by Hedgehog ligand[20]. Hedgehog signaling pathway is widely expressed in a variety of tissues and cells, and

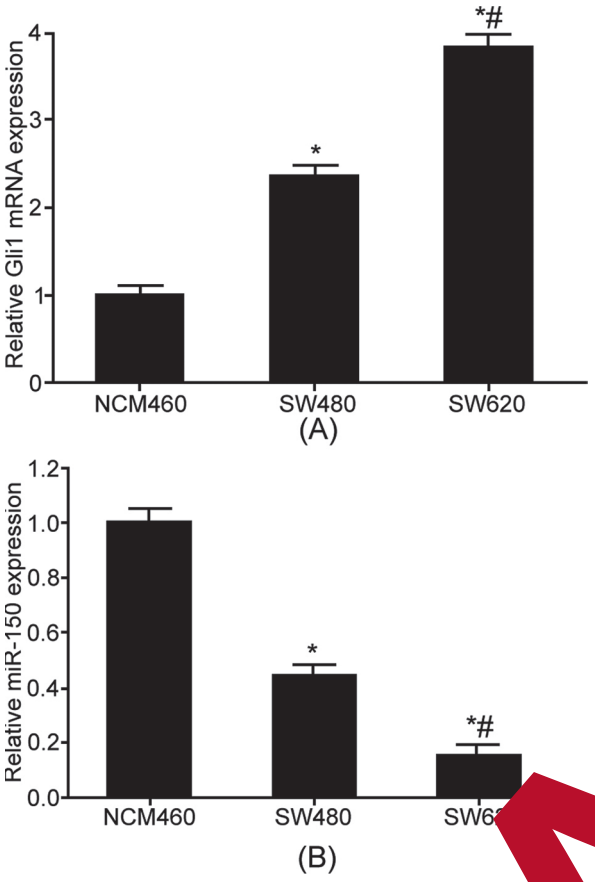


Figure 2. MiR-150 downregulated, while Gli1 overexpr in CRC cells. ***A,*** qRT-PCR detection of Gli1 mR expression. ***B,*** qRT-PCR detection of mi pression. < 0.05, compared with NCM460 cel compared with SW480 cells.

participates in reg g pa cal physiological processes ch as orga elopment and damage [24]. Hedgeho gnaling pathways ar omp of secretory signal protein H ehog, tran brane protein receptor P ed (Ptch) and thened (Smo), middl olecul transcriptional factor protein Gli dow am target genes, etc. When Hed ling p ways is inactive, Ptch combi h Sm suppress its activity, g to ph orylation and ubiquitin, th mpedi entry into the nucleus in ful gth to regulate the transcription and e downstream target genes. Once Hedgehog signaling pathway is activated, ligands bind with transmembrane protein tor Ptch to abrogate the inhibitory effect on o, thus invaliding the degradation of Gli. Gli1 enters the nucleus in the form of a full-length to promote target gene transcription and expression, so as to facilitate cell proliferation, migration, and malignant transformation[25]. Several studies revealed that the aberrant activation of Hedgehog signaling path closely associated with tumorigene progre sion, metastasis, and recurrence as breast cancer[9], pancreatic cancer[10], c cancer[7], and gallbladder carcinoma[8]. As a ber of zinc finger protein famil li1 gen te in human chromosome q13.2-13.3[26]. a member of human protei mily (G Gli2, and Gli3). T bnor upreg lation of Gli1 is an i ort bol o edgehog signaling way e ive ation[27]. It was foun t Gli1 elev elated to multiple t sis, progre n, and metastasis, ludin st cancer[13], esophageal cancer[12], and lung ca l. It was showed that Gli ssion abnorm increased in CRC t e and affected cancer metastasis and progression[14,15]. Mi 50 poses as a downstream tor of my rdial infarction associated t ript (MIA n the development of cardiac Recent research also revealed that mi ownregulated in CRC tissue and as associated with cancer progression, treatfficacy, and prognosis[18,19].

the regulatory relationship between miR-150 and Gli1 mRNA was demonstrated by dual luciferase assay, we further compared miR-150, Gli1 levels among human normal colon epithelial cells and CRC cells. Our data suggested that miR-150 reduction may play a role in elevating Gli1 and promoting tumorigenesis. Ma et al[18] found that miR-150 was significantly declined in CRC tumor tissue compared with that in adjacent normal control by fluorescence *in situ* hybridization and qRT-PCR. Moreover, the survival rate and chemotherapy effect of patients with lower miR-150 expression were worse than that with higher miR-150 level. Sarlinova et al[19] reported that miR-150 expression was reduced in the peripheral blood from CRC patients compared with healthy control. Aheme et al[29] demonstrated that miR-150 was markedly declined in peripheral blood from CRC patients and was correlated with disease progression. In this study, miR-150 level was decreased in CRC cells, revealing that miR-150 may be a tumor suppressor gene of CRC, which was similar with Ma et al[18], Sarlinova et al[19], and Aheme et al[29]. Hong et al[14] exhibited that Gli1 expression was upregulated in CRC tissue compared with that in normal control. Zhang et al[15] discovered

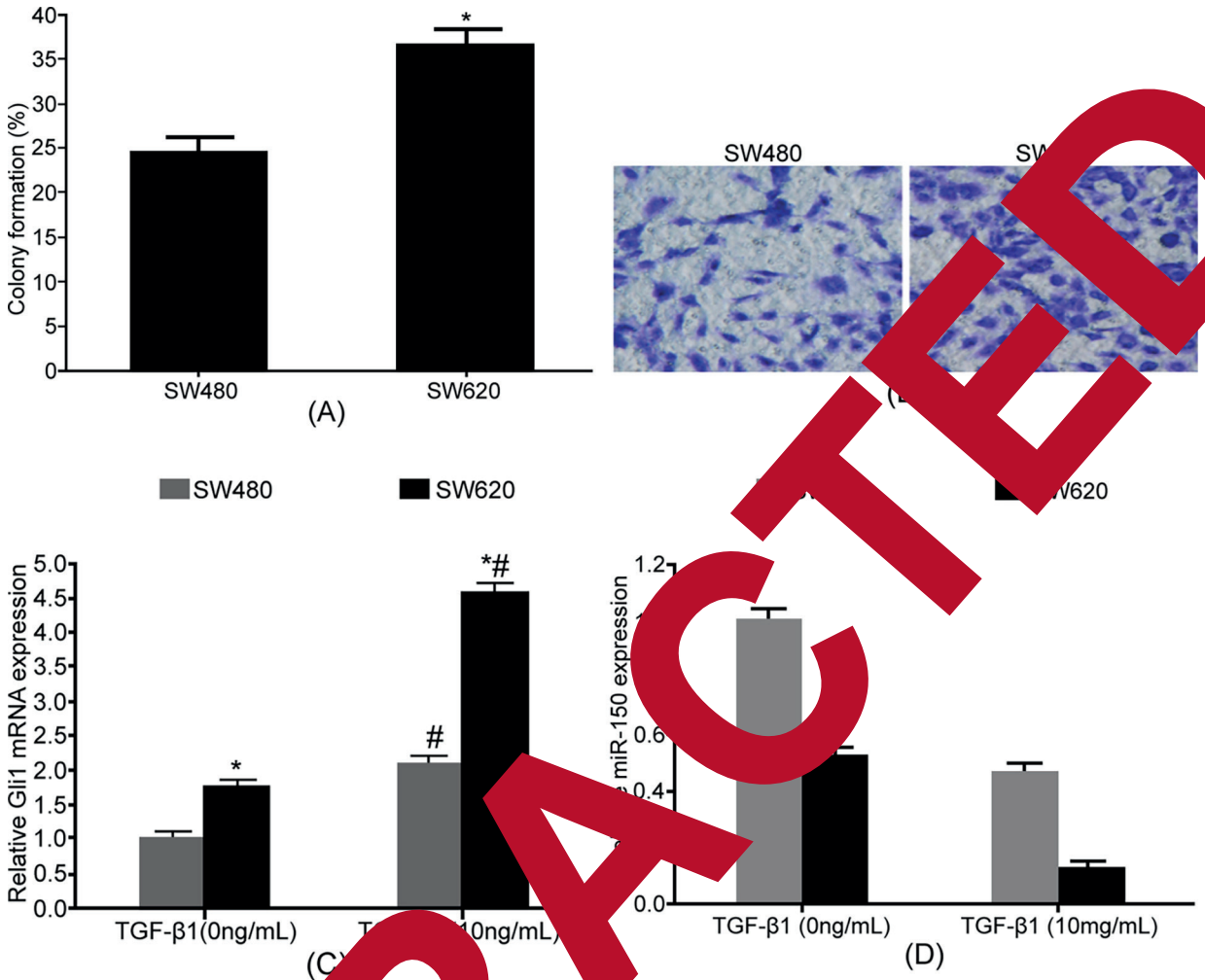
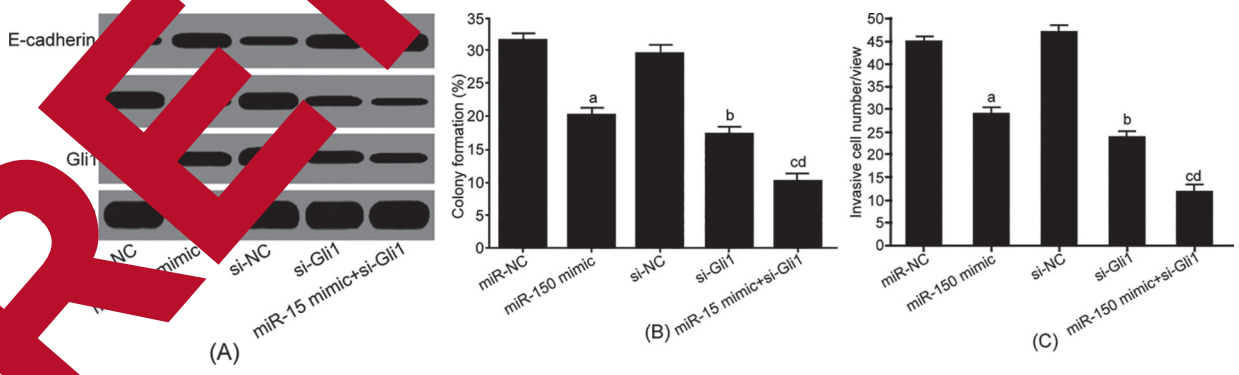


Figure 3. Gli1 increased, wh R-150 re the EMT process of CRC cells. ***A,*** Colony formation assay detection of clone formation. ***B,*** Repres tat ge g assay by Giemsa staining (20×). ***C,*** qRT-PCR detection of Gli1 mRNA expression. ***D,*** qR R de miR-150 expression. *$p < 0.05$, compared with SW480 cells; #$p < 0.05$, compared with TGF-β1 (0 ng/ml)



Fi 4. MiR-150 overexpression downregulated Gli1 level, suppressed EMT process, and attenuated cell invasion in CRC cells. ***A,*** Western blot detection of protein expression. ***B,*** Colony formation assay detection of clone formation. ***C,*** Transwell assay detection of cell invasion. [a]$p < 0.05$, compared with miR-NC; [b]$p < 0.05$, compared with si-NC; [c]$p < 0.05$, compared with miR-150 mimic; [d]$p < 0.05$, compared with si-Gli1 group.

that Gli1 level was increased in CRC tissue compared with that in adjacent normal control, while its level was significantly higher in CRC cells than that in normal colon epithelial cell NCM460. This research found that Gli1 expression was higher in CRC cells than that in normal colon epithelial cells, indicating that Gli1 was a stimulus of colon cancer tumorigenesis, which was in accordance with Hong et al[14] and Zhang et al[15]. Furthermore, Gli1 was upregulated, while miR-150 was reduced in SW620 cells compared with that in SW480 cells. This became more significant in EMT process induced by TGF-β1, suggesting that miR-150 mediated the upregulation of Gli1 and represented a motivator of CRC EMT and invasion. It was proposed that the increase of Snail expression was closely associated with Gli1 in promoting EMT and cancer cell invasion[30]. This study further observed that miR-150 mimic and/or si-Gli1 transfection markedly reduced Gli1 and Snail levels, upregulated E-cadherin expression, and attenuated cell colony formation and invasion, indicating that Gli1 may facilitate SW620 cell EMT and invasion via upregulating Snail. Feng et al[31] reported that miR-150 pla[illegible] tumor suppressor role in CRC. MiR-150 e[illegible] suppressed CRC cell proliferation, blocke[illegible] cycle, restrained migration and invasion, a[illegible] tenuated cell growth *in vivo* by targeting c-M[illegible] expression. Inhibition of miR-1[illegible] tained t[illegible] opposite phenomenon. Wan[illegible] nd that miR-150 weakened the m[illegible] ion a[illegible] nvasion of CRC cell and HCT[illegible] throug[illegible] MUC4 expression. T[illegible] d t[illegible] upregulation of mi[illegible]0 a[illegible] d CRC cell EMT and invasi[illegible] which was[illegible] ccordance with Feng et [illegible] Wang et a[illegible] heng et al[33] demonst[illegible] th[illegible] downregulation of Gli1 mark[illegible]y suppresse[illegible] proliferation and invasion[illegible] CRC cell line[illegible]2 cell, induced cell a[illegible]osis, [illegible]d attenuated drug resistance. Zha[illegible] t al[15] [illegible]ved that the overexpression of Gli[illegible]y facili[illegible]d CRC cell EMT, and enhanc[illegible] migr[illegible], invasion, and metas-*in vi*[illegible]hi[illegible]n of Gli1 alleviated CRC ce[illegible]tastas[illegible]*vo*, which was similar to our res[illegible] This stu[illegible] revealed that the reduction of m[illegible]ion and upregulation of Gli1 pro-[illegible]ed the EMT and invasion of CRC cell. How-[illegible]r, our investigation failed to test miR-150 and [illegible]ynamic expressions in CRC tumor tissues in [illegible]erent stages and metastatic status, which requires further validation to accurately reflect the condition in human body.

Conclusions

Downregulation of miR-150 and elevation of Gli1 facilitate the EMT and invasion[illegible] cells. MiR-150 inhibited the EMT an[illegible]asion[illegible] CRC cells via inhibiting Gli1.

Acknowledgements
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Conflict of Inte[illegible]
The Authors dec[illegible]hat they have [illegible]f[illegible] interests.

Re[illegible]nces

1) [illegible]l, Wu D, Yang C, [illegible]g H, Wang G, Wang T, [illegible]i X. Noncoding RNAs in the development, diagnosis, and p[illegible]osis of colorectal cancer. Transl [illegible]Res 2017; 18[illegible]8-120.
2) [illegible]arley AR, [illegible] H. Epidemiology of colorectal [illegible]er. Int[illegible]l Epidemiol Genet 2016; 7: 105-[illegible]
3) Zhou P, Li B, Liu F, Zhang M, Wang Q, Liu Y, Yao Y, Li D. The epithelial to mesenchymal transition [illegible]l and cancer stem cells: implication for treat-[illegible]t resistance in pancreatic cancer. Mol Cancer 2017; 16: 52.
4) Matysiak M, Kapka-Skrzypczak L, Jodlowska-Jedrych B, Kruszewski M. EMT promoting transcription factors as prognostic markers in human breast cancer. Arch Gynecol Obstet 2017; 295: 817-825.
5) Wu F, Zhang Y, Sun B, McMahon AP, Wang Y. Hedgehog signaling: from basic biology to cancer therapy. Cell Chem Biol 2017; 24: 252-280.
6) Merchant JL, Ding L. Hedgehog signaling links chronic inflammation to gastric cancer precursor lesions. Cell Mol Gastroenterol Hepatol 2017; 3: 201-210.
7) Chaudary N, Pintilie M, Hedley D, Hill RP, Milosevic M, Mackay H. Hedgehog inhibition enhances efficacy of radiation and cisplatin in orthotopic cervical cancer xenografts. Br J Cancer 2017; 116: 50-57.
8) Dixit R, Pandey M, Tripathi SK, Dwivedi AN, Shukla VK. Comparative analysis of mutational profile of sonic hedgehog gene in gallbladder cancer. Dig Dis Sci 2017; 62: 708-714.
9) Habib JG, O'Shaughnessy JA. The hedgehog pathway in triple-negative breast cancer. Cancer Med 2016; 5: 2989-3006.
10) Zheng W, Lu S, Cai H, Kang M, Qin W, Li C, Wu Y. Deguelin inhibits proliferation and migration of human pancreatic cancer cells in vitro targeting hedgehog pathway. Oncol Lett 2016; 12: 2761-2765.

11) Chang KJ, Yang MH, Zheng JC, Li B, Nie W. Arsenic trioxide inhibits cancer stem-like cells via down-regulation of Gli1 in lung cancer. Am J Transl Res 2016; 8: 1133-1143.

12) Yang Z, Cui Y, Ni W, Kim S, Xuan Y. Gli1, a potential regulator of esophageal cancer stem cell, is identified as an independent adverse prognostic factor in esophageal squamous cell carcinoma. J Cancer Res Clin Oncol 2017; 143: 243-254.

13) Kwon YJ, Hurst DR, Steg AD, Yuan K, Vaidya KS, Welch DR, Frost AR. Gli1 enhances migration and invasion via up-regulation of MMP-11 and promotes metastasis in ERalpha negative breast cancer cell lines. Clin Exp Metastasis 2011; 28: 437-449.

14) Hong KD, Lee Y, Kim BH, Lee SI, Moon HY. Expression of GLI1 correlates with expression of lymphangiogenesis proteins, vascular endothelial growth factor C and vascular endothelial growth factor receptor 3, in colorectal cancer. Am Surg 2013; 79: 198-204.

15) Zhang C, Wang Y, Feng Y, Zhang Y, Ji B, Wang S, Sun Y, Zhu C, Zhang D, Sun Y. Gli1 promotes colorectal cancer metastasis in a Foxm1-dependent manner by activating EMT and PI3K-AKT signaling. Oncotarget 2016; 7: 86134-86147.

16) Li C, Deng L, Zhi Q, Meng Q, Qian A, Sang H, Li X, Xia J. MicroRNA-183 functions as an onc[illegible]ne by regulating PDCD4 in gastric cancer. [illegible] cer Agents Med Chem 2016; 16: 447-455.

17) Qiu ZA, He GP. MicroRNA-134 functions as [illegible]-mor suppressor gene in gastric cancer. [illegible] Transl Res 2016; 8: 4320-4328.

18) Ma Y, Zhang P, Wang F, Zhang [illegible] Peng J, Li W, Qin H. miR-150 as a pot[illegible]ker associated with prognosis and [illegible]apeuti[illegible]come in colorectal cancer. Gut [illegible]61: 144[illegible]

19) Sarlinova M, Halasa M, M[illegible]D, [illegible] Slaby O, Mazuchov[illegible]alent[illegible]ank L, Halasova E. miR-21, mi[illegible]1 and miR[illegible]re deregulated in periphe[illegible]od of patient[illegible]colorectal cancer. Ant[illegible] 2016; 36: 5[illegible]3454.

20) Song Z, [illegible] B, Lu [illegible]iang X, Li P, Chen L. Metfor[illegible] suppresses [illegible]xpression of Sonic hedg[illegible]g in gastric canc[illegible]ls. Mol Med Rep 20[illegible]5: 1909-1915.

21) [illegible]uca A, [illegible]ato V, Fuca E, Parmigiani E, Buffo [illegible]o K, [illegible]ic hedg[illegible]hog patterning during cere[illegible]elopmen[illegible]ell Mol Life Sci 2016; 73: 291[illegible]

[illegible]gh BF, [illegible]akagawa N, Donaldson A, Weavver CV, Gar[illegible], Garry DJ. Hedgehog signaling during appendage development and regeneration. Genes (Basel) 2015; 6: 417-435.

23) Sriperumbudur A, Breitzig M, Lockey R, Kolliputi N. Hedgehog: the key to maintaining ad[illegible] pair and regeneration. J Cell Co[illegible] Sig[illegible] 2017; 11: 95-96.

24) Wang Y, Lu P, Zhao D, Sheng [illegible]rgeting the hedgehog signaling pathway fo[illegible]ac repair and regeneration. Herz 20[illegible]42: 60[illegible]

25) Gu D, Schlotman KE, X[illegible]Deciphering [illegible]l of hedgehog signalin[illegible] pancr[illegible]atic ca[illegible] Biomed Res 2016; [illegible]53-360[illegible]

26) Carpenter RL, Lo[illegible]Hedg[illegible]g pathw[illegible]y and GLI1 isoforms [illegible]h[illegible]cer. Di[illegible]Med 2012; 13: 10[illegible]

27) Marechal [illegible]het JB, Calom[illegible]ter P, Delpero JR, [illegible]Cros J, Bar[illegible]pas A, Puleo F, Mo[illegible]G, [illegible]P, Louvet C, Paye F, Bacheliер P, Le[illegible]treut YP, [illegible]t JC, Sauvanet A, Andre T, Sa[illegible]L, Deviere J, [illegible]F, Van Laethem JL. Sonic [illegible]og and Gli1 exp[illegible]on predict outcome in [illegible]sected pancreatic ade[illegible]ocarcinoma. Clin Cancer Res 2015; [illegible]: 1215-1224.

Zhu XH, Yuan [illegible]Rao SL and Wang P. LncRNA MI[illegible]T enhances [illegible]diac hypertrophy partly through [illegible]nging m[illegible]0. Eur Rev Med Pharmacol Sci [illegible]-3660.

29) Aherne [illegible], Madden SF, Hughes DJ, Pardini B, Naccarati A, Levy M, Vodicka P, Neary P, Dowling P, [illegible]s M. Circulating miRNAs miR-34a and miR-[illegible]associated with colorectal cancer progression. BMC Cancer 2015; 15: 329.

30) Min S, Xiaoyan X, Fanghui P, Yamei W, Xiaoli Y, Feng W. The glioma-associated oncogene homolog 1 promotes epithelial--mesenchymal transition in human esophageal squamous cell cancer by inhibiting E-cadherin via Snail. Cancer Gene Ther 2013; 20: 379-385.

31) Feng J, Yang Y, Zhang P, Wang F, Ma Y, Qin H, Wang Y. miR-150 functions as a tumour suppressor in human colorectal cancer by targeting c-Myb. J Cell Mol Med 2014; 18: 2125-2134.

32) Wang WH, Chen J, Zhao F, Zhang BR, Yu HS, Jin HY, Dai JH. MiR-150-5p suppresses colorectal cancer cell migration and invasion through targeting MUC4. Asian Pac J Cancer Prev 2014; 15: 6269-6273.

33) Cheng J, Deng R, Wu C, Zhang P, Wu K, Shi L, Liu X, Bai J, Deng M, Gao J, Shuai X, Wang G, Tao K. Inhibition of SALL4 suppresses carcinogenesis of colorectal cancer via regulating Gli1 expression. Int J Clin Exp Pathol 2015; 8: 10092-10101.