WITHDRAWN ARTICLE

WITHDRAWN: LncRNA EGOT regulates the proliferation and apoptosis of colorectal cancer by miR-33b-5p/CROT axis

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LncRNA EGOT regulates the proliferation and apoptosis of colorectal cancer by miR-33b-5p/CROT axis

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Abstract

Accumulating researches have proved that long noncoding RNAs (lncRNAs) regulate a variety of cellular processes during cancer progression. However, the detailed function of most lncRNAs in

1

23   colorectal cancer (CRC) remains mostly unknown. This study was aimed

24   at exploring the specific role of lncRNA EGOT in CRC. Data from this

25   study revealed that EGOT expression was obviously upregulated in CRC

26   tissues and cell lines, and high EGOT expression indicated poor overall

27   survival of CRC patients. Besides, functional assays proved that EGOT

28   knockdown inhibited cell proliferation and promoted cell apoptosis in

29   CRC. Then, subsequent molecular mechanism assays uncovered that

30   EGOT could bind with miR-33b-5p and negatively regulate miR-33b-5p

31   expression. Additionally, CROT was a downstream target of miR-33b-5p.

32   Further, rescued-function assays suggested that the suppressive influence

33   of EGOT depletion on CRC progression was reversed by miR-33b-5p

34   inhibition or CROT overexpression. In conclusion, lncRNA EGOT

35   mediates the tumor-facilitating part in CRC via miR-33b-5p/CROT

36   pathway.

37   **Key words:** EGOT; CRC; miR-33b-5p; CROT.

38

39   **Introduction**

40   Colorectal cancer (CRC) is a kind of frequent malignant cancer with poor

41   clinical outcome (1). The only hope of treating precancerous CRC might

42   be the radical surgery (2). However, even after the surgery, radiation and

43   chemotherapy still have little effect (3). Previous researches have

44   indicated that some molecular markers are associated with prognosis, but

45   the pathogenesis of CRC is still unclear (4). Hence, it is an urgent thing to

46   identify potential biomarkers related to cancer progression, which can

47   help develop new treatments for CRC.

48   Long noncoding RNAs (lncRNAs) are recognized as a group of RNA

49   molecules which are longer than 200 nucleotides and lack any open

50   reading frame to encode protein. Besides, they have been previously

51   manifested as crucial regulators in diverse tumors (5, 6). Specifically,

52   lncRNA SPRY4-IT1 contributes to the tumorigenesis of gastric cancer (7);

53   lncRNA PANDAR predicts poor prognosis and is involved in

54   tumorigenesis in hepatocellular carcinoma (8); and lncRNA LUNAR1

55   facilitates cell proliferation and high expression of LUNAR1 leads to

56   poor prognosis in diffuse large B-cell lymphoma (9). LncRNA EGOT is

57   backward-looking at the level of nucleotide and was initially found to be

58   implicated in the development of eosinophils and expressed in mature

59   eosinophils (10). In addition, EGOT has been revealed to have a high

60   expression in bone marrow and function in the development of bone

61   marrow hematopoietic stem cells (11). In recent years, the close

62   relationship between abnormally expressed EGOT and tumorigenesis has

63   been clarified in many cancers (11-15). The findings of these researches

64   imply that dysregulation of EGOT is tightly associated with prognosis

65   and that EGOT plays significant role in the occurrence and development

66   of tumors. However, the specific role of EGOT in CRC is largely

67   unknown.

68   This study was the first attempt to investigate the underlying function and

69   mechanism of EGOT in CRC, aiming to shed new lights on CRC

70   treatment.

71

72   **Materials and methods**

73   **Clinical specimens**

74   The 70 pairs CRC tissue specimens and matched non-tumor tissue

75   specimens were collected from CRC patients from 2013 and 2018. The

76   ethical approval of this study was obtained from the Ethics Committee of

77   Harbin Medical University Cancer Hospital. All the participants didn't

78   receive any treatment and they all signed the informed consents prior to

79   this study. All clinical samples were instantly frozen in liquid nitrogen

80   and further stored at -80°C.

81

82   **Cell lines**

83   Human normal cell line NCM460 and four CRC cell lines including

84   SW480, SW620, HCT15 and HT29, were cultivated continuously in

85   DMEM (Gibco, Grand Island, NY, USA) at 37°C and 5% CO_2. All cell

86   lines were purchased from the American Type Culture Collection (ATCC;

87   Rockville, Maryland, USA). Culture medium was added with 10% fetal

88   bovine serum (FBS; Gibco) and antibiotics as supplements.

89

Quantitative real-time polymerase chain reaction (qRT-PCR)

Extraction of total RNAs from SW620 and HT29 cells or tissue samples

were performed using TRIzol Regent (Invitrogen, Carlsbad, CA, USA). 1

µg of sample was reversely transcribed into cDNA for conducting qPCR

in the presence of SYBR® Premix Ex Taq™ II (Takara, Tokyo, Japan) on

the Step-One Plus Detection System (Applied Biosystems, Foster City,

CA, USA). RNA level was calculated by the comparative $2^{-\Delta\Delta Ct}$ method.

The sequences of primers were listed in Supplementary Table 1.

Cell transfection

CRC cells (SW620 and HT29) were seeded in the 6-well and grown in an

incubator with CO_2 at 37°C. The two specific shRNAs to EGOT

(sh-EGOT#1/2), CROT (sh-CROT#1/2) and control shRNAs (sh-NC), as

well as pcDNA3.1/CROT (with empty pcDNA3.1 vector as control),

were all produced at RiboBio (Guangzhou, China). Moreover,

miR-33b-5p mimics and NC mimics, miR-33b-5p inhibitor and NC

inhibitor, were bought from Genechem (Shanghai, China). Cell

transfection was performed for 48 h in the presence of Lipofectamine

2000 reagent (Invitrogen). The shRNA sequences were shown in

Supplementary Table 2.

Colony formation assay

Transfected CRC cell lines were plated into 6-well plates (1×10^3/100 μL) at 37°C with 5% CO_2 for 2 weeks. Colonies were rinsed in phosphate-buffered saline (PBS), fixed in 5% paraformaldehyde and labeled with 1 mL of 0.1% crystal violet solution. After counting manually, visible colonies were recorded.

TUNEL assay

CRC cells were transfected with indicated plasmids and harvested for TUNEL assay. Fixed cells in 1% formaldehyde were subjected to 0.2% Triton X-100 for permeabilization and dUTP-end labeling kit (Clontech, Mountain View, CA, USA). Cells were counterstained with DAPI and analyzed by fluorescence microscope (NIKON, Tokyo, Japan).

Western blot

Cells were seeded into 6-well plates prior to being collected for western blot analysis. The total proteins were isolated from the cell lines using RIPA buffer reagent (Thermo Fisher Scientific, Rochester, New York, USA) separated with 10% SDS-PAGE, and transferred onto PVDF membranes on ice overnight. Bovine serum albumin (BSA, 5%; Beyotime, Shanghai, China) was used to block membranes for 2 h at room temperature. Afterwards, membranes were subjected to primary

6

133 antibodies and secondary antibodies in succession. Primary antibodies

134 including anti-Bcl-2 (ab692), anti-Bax (ab182733), anti-cleaved

135 caspase-3 (ab2302), anti-total caspase-3 (ab2302), anti-CROT (ab175450),

136 anti-Tubulin (ab210797) and anti-GAPDH (ab22555), together with

137 HRP-labeled secondary antibodies were all acquired from Abcam

138 (Cambridge, MA, USA). Subsequent to washing in PBS, bands were

139 visualized with enhanced chemiluminescence (ECL) system (Bio-Rad lab,

140 Hercules, CA, USA).

141

142 **Subcellular fractionation**

143 In line with the manufacturer's recommendations of PARIS™ Kit

144 (Invitrogen), subcellular fractionation was assayed in SW620 and HT29

145 cells. The cell lysates from cell fractionation buffer were first centrifuged

146 and then mixed with cell disruption buffer. The levels of EGOT in

147 cytoplasmic and nuclear fractions were determined by qRT-PCR, with

148 GADPH and U6 as cytoplasmic and nuclear, respectively.

149

150 **Dual-luciferase reporter vector analysis**

151 For EGOT and CROT 3'UTR luciferase analysis, the pmirGLO

152 Dual-Luciferase reporter constructs containing wild type or mutant

153 miR-33b-5p interacting sequences were formed and termed as

154 EGOT-WT/Mut and CROT-WT/Mut. SW620 or HT29 cells in 24-well

7

155 plates were co-transfected with above reporter constructs and indicated

156 transfection plasmids for 48 h, followed by analysis by the Dual

157 Luciferase Assay System (Promega, Madison, WI, USA). Firefly

158 luciferase activity was normalized to the corresponding Renilla luciferase

159 activity.

160

161 **RNA immunoprecipitation (RIP)**

162 Using EZ-Magna RIP Kit (Millipore, Billerica, MA, USA), RIP assay

163 was carried out in CRC cells. SW620 and HT29 cells were washed in

164 ice-cold PBS and lysed in RIP lysis buffer. Human anti-Ago2 antibody

165 and nonspecific control normal IgG antibody (Millipore) were used for

166 immunoprecipitation. RIP lysates were cultured with magnetic beads all

167 night and then precipitated RNAs were purified for qRT-PCR analysis.

168

169 **RNA pull-down assay**

170 The wild type or mutant CROT sequences which contained the possible

171 miR-33b-5p binding sites were obtained and biotinylated into

172 Bio-CROT-WT/Mut. Cell extracts from RIPA lysis buffer were treated

173 with Bio-CROT-WT/Mut or Bio-NC. After adding magnetic beads, the

174 complex was centrifuged and evaluated by qRT-PCR.

175

176 **Statistical analyses**

177   Graph generation and data analyses in this study were both disposed via

178   PRISM 6 (GraphPad, San Diego, CA, USA). Besides, data were showed

179   as the mean ± SD of three independent assays. Significant differences

180   between groups were assayed through Student's t test or ANOVA, with

181   threshold of probability (p-value) less than 0.05.

182

183   **Results**

184   **Downregulation of EGOT inhibited cell proliferation and promoted**

185   **cell apoptosis in CRC**

186   To make the exploration of the underlying role that EGOT played in CRC,

187   we first detected its expression status in tissue samples obtained from

188   CRC patients. And the data from qRT-PCR uncovered that EGOT was

189   highly expressed in CRC tissue specimens versus peri-tumor specimens

190   (Fig. 1A). Then clinical data revealed that high expression of EGOT

191   resulted in low survival rate of CRC patients (Fig. 1B). Next, the

192   expression of EGOT was examined in the normal NCM460 cells and four

193   CRC cell lines by qRT-PCR. The result showed that EGOT expression

194   was relatively high in CRC cell lines, particularly in SW620 and HT29

195   cells (Fig. 1C). Therefore, SW620 and HT29 cells were selected for the

196   following experiments. After knocking down EGOT in SW620 and HT29

197   cells by transfection with sh-EGOT#1/2 (Fig. 1D), cell proliferation

198   ability was weakened (Fig. 1E). Conversely, data from TUNEL assay

9

199 revealed that knockdown of EGOT facilitated the apoptosis of SW620

200 and HT29 cells (Fig. 1F). Besides, western blot analysis demonstrated

201 that downregulation of EGOT decreased the expression of Bcl-2 whereas

202 increased the expression of Bax and cleaved caspase-3, further validating

203 that silenced EGOT could induce the apoptosis of SW620 and HT29 cells

204 (Fig. 1G). All these data clarified that EGOT was overexpressed in CRC

205 tissues and cells, and EGOT depletion suppressed cell proliferation but

206 promoted cell apoptosis in CRC.

207 **MiR-33b-5p combined with EGOT**

208 Afterwards, we aimed to study the mechanism of EGOT in CRC, the

209 subcellular fractionation assay was first implemented to find out the

210 subcellular location of EGOT. Interestingly, the result showed that EGOT

211 was mostly located in the cytoplasm of SW620 and HT29 cells (Fig. 2A),

212 implying that EGOT was involved in the posttranscriptional regulation of

213 gene expression. After utilizing online starBase, 7 miRNAs which could

214 possibly bind with EGOT were predicted. Then, data from qRT-PCR

215 revealed that only the expression of miR-33b-5p was found affected most

216 by knockdown of EGOT (Fig. 2B). To further investigate the interaction

217 between miR-33b-5p and EGOT, starBase was applied again to predict

218 the binding sites between EGOT and miR-33b-5p (Fig. 2C). After

219 elevating miR-33b-5p expression in SW620 and HT29 cells by

220 transfection with miR-33b-5p mimics (Fig. 2D), the luciferase activity of

221   EGOT-WT reporter was decreased a lot. Nevertheless, when the binding

222   sequences of EGOT were mutated, the luciferase activity of EGOT-Mut

223   reporter showed no clear changes between two groups (Fig. 2E). This

224   indicated that EGOT could interact with miR-33b-5p at predicted sites.

225   Moreover, qRT-PCR analysis demonstrated that the expression of

226   miR-33b-5p was signally elevated in CRC cell lines when compared with

227   the normal cell line (Fig. 2F). Further, we observed that the proliferation

228   ability of SW620 and HT29 cells was obviously decreased after

229   miR-33b-5p was overexpressed (Fig. 2G). On the contrary, upregulation

230   of miR-33b-5p could promote the apoptosis of SW620 and HT29 cells

231   (Fig. 2H). Taken together, EGOT targeted the tumor-suppressive

232   miR-33b-5p in CRC.

233   **CROT was the target of miR-33b-5p**

234   Based on the above findings, we proceeded to find the targets of

235   miR-33b-5p by utilizing starBase. With the help of five bioinformatics

236   tools (miRmap, PITA, TargetScan, PicTar and RNA22), two possible

237   candidate targets of miR-33b-5p were screened out (Fig. 3A). Then, it

238   was exhibited that under the condition of EGOT knockdown, CROT

239   expression was downregulated while NAA15 expression was not

240   influenced in SW620 and HT29 cells (Fig. 3B). Thus, CROT was chosen

241   for further analysis. Later, the binding sites between CROT and

242   miR-33b-5p were predicted by starBase (Fig. 3C). Afterwards, the

243   luciferase reporter assay suggested that elevating miR-33b-5p expression

244   decreased the luciferase activity of CROT-WT reporter but not that of

245   CROT-Mut reporter (Fig. 3D). Furthermore, RIP and RNA pull down

246   assays further proved that CROT could bind with miR-33b-5p (Fig.

247   3E-F).More importantly, it was proved that the suppressive effect of

248   silenced EGOT on the mRNA and protein levels of CROT was reversed

249   by miR-33b-5p inhibition (Fig. 3G-H). These data showed that EGOT

250   regulated the expression of CROT in CRC by competitively binding with

251   miR-33b-5p. Then, the expression of CROT was found upregulated in

252   CRC cell lines (Fig. 3I). Moreover, cell proliferation capability was

253   attenuated whereas the apoptosis capability was enhanced in SW620 and

254   HT29 cells with CROT depletion (Fig. 3J-L). Briefly, CROT was the

255   target of miR-33b-5p and CROT functioned as a tumor promoter in CRC.

256   **LncRNA EGOT regulated the proliferation and apoptosis of CRC by**

257   **targeting miR-33b-5p/CROT axis**

258   After validating the existence of EGOT/miR-33b-5p/CROT network in

259   CRC, we further studied the influence of this competing endogenous

260   RNA (ceRNA) mechanism on CRC cell proliferation and apoptosis. Prior

261   to rescued-function assays, we conducted qRT-PCR assay and obtained a

262   satisfactory efficiency of CROT overexpression (Fig. 4A). Expectedly,

263   we unveiled that miR-33b-5p inhibition or CROT upregulation could

264   rescue the suppressive influence of EGOT depletion on cell proliferation

265 (Fig. 4B). Likewise, the increased cell apoptosis rate caused by EGOT

266 silence was reversed by inhibiting miR-33b-5p expression or

267 overexpressing CROT (Fig. 4C), while such phenomenon was further

268 testified by western blot data (Fig. 4D). To sum up, EGOT regulated CRC

269 cell proliferation and apoptosis via miR-33b-5p/CROT axis.

270

271 **Discussion**

272 CRC is the leading cause of cancer-related mortality worldwide.

273 Accumulating evidence has verified that lncRNA is implicated in the

274 progression of many malignant tumors, including CRC (16-19). Disorder

275 of EGOT is a common phenomenon in different kinds of cancers, such as

276 breast cancer (11), gastric cancer (14) and renal cell carcinoma (12). As

277 far as we know, few studies have focused on the specific role of lncRNA

278 EGOT in CRC. Therefore, further study of EGOT in CRC may do some

279 help for the treatment of this fatal disease. This study first revealed the

280 expression pattern of EGOT in CRC and further explored the impact of

281 EGOT on CRC cell proliferation and apoptosis, along with its underlying

282 mechanism.

283 In our study, EGOT was expressed at high levels in CRC tissues and cells,

284 and EGOT predicted poor prognosis of CRC patients, indicating that

285 EGOT might be a possible biomarker for CRC diagnosis. Next, we

286 studied the impact of EGOT on CRC cell proliferation and apoptosis.

13

287   Data from loss-of-function assays confirmed the tumor-accelerating effect

288   of EGOT on CRC tumorigenesis. Moreover, Bcl-2 and Bax are essential

289   regulators for the activity of cell apoptosis. Accumulating studies have

290   shown that lncRNAs regulate the expression of Bcl-2 and Bax in various

291   cancers. Yin Z et al uncovered that PPMD1 depletion could increase the

292   protein level of Bax and decrease the protein level of Bcl-2 expression,

293   thus inducing cell apoptosis in colon cancer (20). Wang AH et al

294   elucidated that lncRNA PCAT-1 could regulate Bax and Bcl-2 expression

295   and facilitate cell apoptosis in CRC (21). In this paper, we proved that

296   EGOT depletion decreased Bcl-2 expression and enhanced Bax

297   expression in CRC and induced cell apoptosis in CRC. Besides, several

298   previous findings unveiled that EGOT promoted tumorigenesis in gastric

299   cancer through activating Hedgehog pathway (22), which provided

300   another way for the potential downstream pathway for EGOT in

301   regulating CRC.

302   Since ceRNA is a typical post-transcriptional pattern and EGOT was

303   mainly distributed in cytoplasm of CRC cells, we assumed that EGOT

304   served as a ceRNA via sponging certain miRNA to regulate CRC cells.

305   Via a range of molecular mechanism assays and rescue tests, we verified

306   that EGOT competitively bound with miR-33b-5p to enhance CROT

307   expression, thus facilitating CRC cell proliferation. Interestingly, Yang X

308   et al. revealed that miR-33b-5p could sensitize gastric cancer cells to

14

309 chemotherapy drugs via targeting HMGA2 (23). Moreover, Zhao M et al.

310 previously disclosed the existence of ceRNA network containing

311 miR-33b-5p   in   prostate   cancer,   and   clarified   that

312 CUL4B/miR-33b/C-MYC axis contributed to the progression of prostate

313 cancer (24). However, CROT was scarcely researched in cancers.

314 Previously, Chen X et al. suggested that FECH/CROT axis could predict

315 recurrence of prostate cancer (25). Besides, Hage-Sleiman R et al.

316 indicated that CROT was involved in oxidative stress response in

317 leukemia cells of Molt-4 (26).

318 Further, lncRNA-associated ceRNA pattern in CRC has been widely

319 reported. Yu C et al. unveiled that lncRNA AC009022.1 could promote

320 CRC cell proliferation, migration and invasion via promotion on

321 ACTR3B expression through miR-497-5p suppression (27). And Wang Y

322 et al. suggested that lncRNA TTN-AS1 enhanced KLF15 expression via

323 acting as a sponge of miR-376a-3p, therefore promoting CRC progression

324 (28). In addition, Wang Y et al. manifested that upregulation of lncRNA

325 CCAT5 could boost CRC cell growth, invasion and metastasis through

326 activation of STAT3 (29). Consistently, our study further provided the

327 evidence of the existence of ceRNA mechanism in CRC and

328 EGOT/miR-33b-5p/CROT axis was firstly confirmed to influence CRC

329 cell proliferation and apoptosis.

330 On the whole, present study unveiled a novel ceRNA pattern in which

331 EGOT competitively bound with miR-33b-5p to upregulate CROT

332 expression, therefore promoting carcinogenesis in CRC. This finding

333 might provide novel promising biomarkers for CRC treatment.

334

335 **Ethical Standards**

336 The ethical approval of tissue samples was obtained from the Ethics

337 Committee of Harbin Medical University Cancer Hospital. All the

338 participants signed the informed consents.

339

340 **Funding**

341 No funding was received.

342

343 **Authors' contribution**

344 This research was designed mainly by Yin Ni and most of the

345 experiments were accomplished by Yin Ni and Chunbo Li; the

346 experimental data was acquired by Chen Bo; the data was analyzed and

347 interpreted by Bomiao Zhang; figures and tables was completed by

348 Yanlong Liu and Xuefeng Bai; the writing and revision of the manuscript

349 was finished by Bingbing Cui and Peng Han.

350

351 **Conflict of interest**

352 None.

16

353

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356

References

1. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet (London, England). 2005;365(9454):153-65.

2. Kjeldsen BJ, Kronborg O, Fenger C, Jorgensen OD. A prospective randomized study of follow-up after radical surgery for colorectal cancer. The British journal of surgery. 1997;84(5):666-9.

3. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996;87(2):159-70.

4. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nature reviews Cancer. 2009;9(7):489-99.

5. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes & development. 2009;23(13):1494-504.

6. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629-41.

7. Peng W, Wu G, Fan H, Wu J, Feng J. Long noncoding RNA SPRY4-IT1 predicts poor patient prognosis and promotes tumorigenesis in gastric cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(9):6751-8.

8. Peng W, Fan H. Long non-coding RNA PANDAR correlates with poor prognosis and promotes tumorigenesis in hepatocellular carcinoma. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2015;72:113-8.

9. Peng W, Feng J. Long noncoding RNA LUNAR1 associates with cell proliferation and predicts a poor prognosis in diffuse large B-cell lymphoma. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2016;77:65-71.

10. Wagner LA, Christensen CJ, Dunn DM, Spangrude GJ, Georgelas A, Kelley L, et al. EGO, a novel, noncoding RNA gene, regulates eosinophil granule protein transcript expression. Blood. 2007;109(12):5191-8.

11. Xu SP, Zhang JF, Sui SY, Bai NX, Gao S, Zhang GW, et al. Downregulation of the long noncoding RNA EGOT correlates with malignant status and poor prognosis in breast cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(12):9807-12.

12. Jin L, Quan J, Pan X, He T, Hu J, Li Y, et al. Identification of lncRNA EGOT as a tumor suppressor in renal cell carcinoma. Molecular medicine reports. 2017;16(5):7072-9.

13. Kolenda T, Kopczynska M, Guglas K, Teresiak A, Blizniak R, Lasinska I, et al. EGOT lncRNA in head and neck squamous cell carcinomas. Polish journal of pathology : official journal of the Polish Society of Pathologists. 2018;69(4):356-65.

14. Peng W, Wu J, Fan H, Lu J, Feng J. LncRNA EGOT Promotes Tumorigenesis Via Hedgehog Pathway in Gastric Cancer. Pathology oncology research : POR. 2019;25(3):883-7.

15. Wu Y, Liang S, Xu B, Zhang R, Zhu M, Zhou W, et al. Long noncoding RNA eosinophil granule ontogeny transcript inhibits cell proliferation and migration and promotes cell apoptosis in human glioma. Experimental and therapeutic medicine. 2017;14(4):3817-23.

16. Lu MH, Tang B, Zeng S, Hu CJ, Xie R, Wu YY, et al. Long noncoding RNA BC032469, a novel competing endogenous RNA, upregulates hTERT expression by sponging miR-1207-5p and promotes proliferation in gastric cancer. Oncogene. 2016;35(27):3524-34.

17. Pyfrom SC, Luo H, Payton JE. PLAIDOH: a novel method for functional prediction of long non-coding RNAs identifies cancer-specific LncRNA activities. BMC genomics. 2019;20(1):137.

18. Wan L, Sun M, Liu GJ, Wei CC, Zhang EB, Kong R, et al. Long Noncoding RNA PVT1 Promotes Non-Small Cell Lung Cancer Cell Proliferation through Epigenetically Regulating LATS2 Expression. Molecular cancer therapeutics. 2016;15(5):1082-94.

19. Zhao L, Sun H, Kong H, Chen Z, Chen B, Zhou M. The Lncrna-TUG1/EZH2 Axis Promotes Pancreatic Cancer Cell Proliferation, Migration and EMT Phenotype Formation Through Sponging Mir-382. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2017;42(6):2145-58.

20. Yin Z, Yi J, Nie T, Yang Z, Ding N, Du SX, et al. Silencing of PPMD1 inhibits proliferation of human colon cancer cells via induction of apoptosis and cell cycle arrest. Journal of BUON : official journal of the Balkan Union of Oncology. 2019;24(4):1464-9.

21. Wang AH, Fan WJ, Fu L, Wang XT. LncRNA PCAT-1 regulated cell proliferation, invasion, migration and apoptosis in colorectal cancer through targeting miR-149-5p. European review for medical and pharmacological sciences. 2019;23(19):8310-20.

22. Peng W, Wu J, Fan H, Lu J, Feng J. LncRNA EGOT Promotes Tumorigenesis Via Hedgehog Pathway in Gastric Cancer. Pathology & Oncology Research. 2019;25(3):883-7.

23. Yang X, Zhao Q, Yin H, Lei X, Gan R. MiR-33b-5p sensitizes gastric cancer cells to chemotherapy drugs via inhibiting HMGA2 expression. Journal of drug targeting. 2017;25(7):653-60.

24. Zhao M, Qi M, Li X, Hu J, Zhang J, Jiao M, et al. CUL4B/miR-33b/C-MYC axis promotes prostate cancer progression. The Prostate. 2019;79(5):480-8.

25. Chen X, Hu L, Wang Y, Sun W, Yang C. Single Cell Gene Co-Expression Network Reveals FECH/CROT Signature as a Prognostic Marker. Cells. 2019;8(7).

26. Hage-Sleiman R, Bahmad H, Kobeissy H, Dakdouk Z, Kobeissy F, Dbaibo G. Genomic alterations during p53-dependent apoptosis induced by gamma-irradiation of Molt-4 leukemia cells. PloS one. 2017;12(12):e0190221.

27. Yu C, Zhang F. LncRNA AC009022.1 enhances colorectal cancer cells proliferation, migration, and invasion by promoting ACTR3B expression via suppressing miR-497-5p. Journal of cellular biochemistry. 2019.

28. Wang Y, Jiang F, Xiong Y, Cheng X, Qiu Z, Song R. LncRNA TTN-AS1 sponges miR-376a-3p to promote colorectal cancer progression via upregulating KLF15. Life sciences. 2019:116936.

29. Wang Y, Yan XL, Tian SK. Downregulating long non-coding RNA CCAT5 inhibits tumor growth, invasion and metastasis in colorectal cancer through suppressing STAT3. European review for medical and pharmacological sciences. 2019;23(18):7899-904.

Figure legends

Figure 1 Down-regulation of EGOT inhibited cell proliferation and

434 **promoted cell apoptosis in CRC.**

435 A. EGOT expression CRC tissue samples and peri-tumor samples was

436 detected via qRT-PCR.

437 B. The association between EGOT expression and overall survival of

438 CRC patients was analyzed via Kaplan-Meier analysis.

439 C. The expression of EGOT was tested in the normal NCM460 cells and

440 four CRC cell lines by qRT-PCR.

441 D. The efficiency of EGOT knockdown in SW620 and HT29 cells was

442 examined via qRT-PCR.

443 E. Colony formation assay was done to test the effect of EGOT

444 knockdown on cell proliferation.

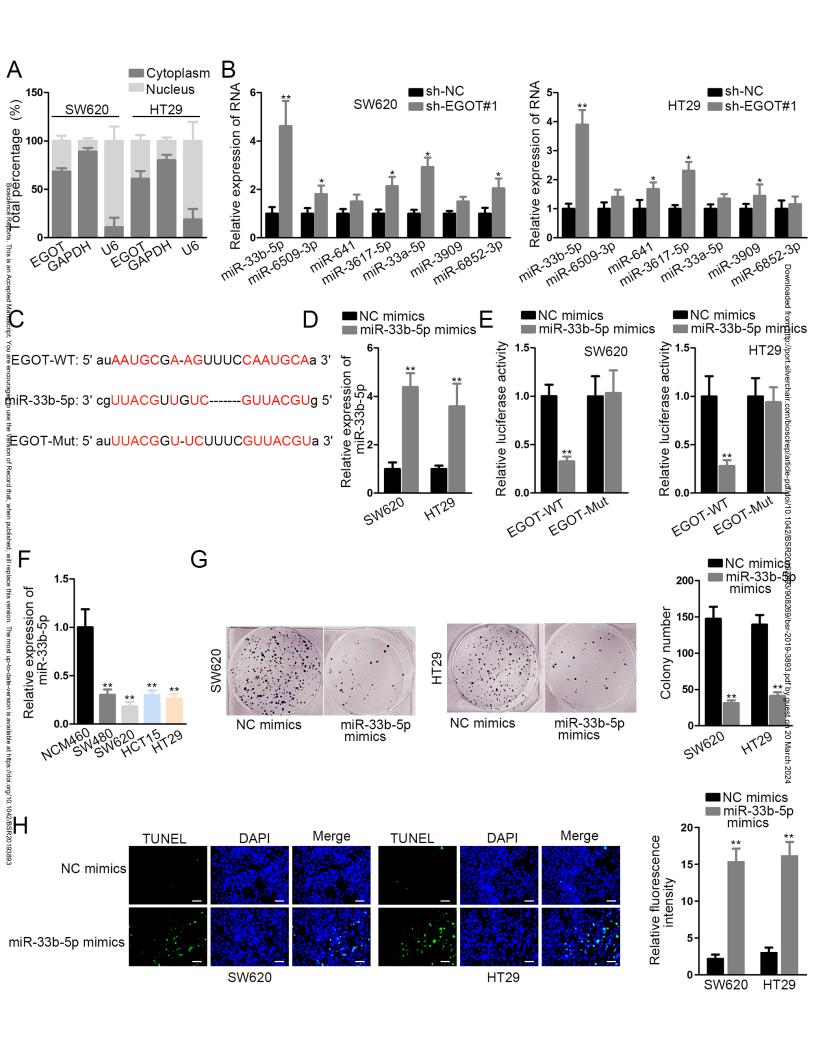445 F. TUNEL assay was implemented to examine the impact of EGOT

446 depletion on cell apoptosis. Scale bar: 200 μm.

447 G. Western blot analysis was used to analyze the influence of EGOT

448 deficiency on the expression of apoptosis-related proteins. [**]P < 0.01.

449 **Figure 2 MiR-33b-5p combined with EGOT.**

450 A. The subcellular fractionation assay was used to determine the

451 subcellular location of EGOT in SW620 and HT29 cells.

452 B. qRT-PCR was used to test the effect of silenced EGOT on the

453 expression of seven candidate miRNAs in SW620 and HT29 cells.

454 C. The binding sites between EGOT and miR-33b-5p were predicted by

455 starBase.

19

456   D. The efficiency of miR-33b-5p upregulation was examined by

457   qRT-PCR.

458   E. Luciferase reporter assay was done to confirm the interaction between

459   EGOT and miR-33b-5p.

460   F. The expression of miR-33b-5p in CRC cell lines compared to NCM460

461   cells was examined via qRT-PCR.

462   G. The proliferation of SW620 and HT29 cells transfected with

463   miR-33b-5p mimics or NC mimics was evaluated via colony formation

464   assay.

465   H. The apoptosis of transfected cells was analyzed via TUNEL assay.

466   Scale bar: 200 μm. $^{*}P < 0.05$, $^{**}P < 0.01$.

467   **Figure 3 CROT was the target of miR-33b-5p.**

468   A. Target genes of miR-33b-5p were predicted by starBase.

469   B. The expression of CROT and NAA15 in SW620 and HT29 cells

470   transfected with sh-NC and sh-EGOT#1 was detected by qRT-PCR.

471   C. The binding sites between CROT and miR-33b-5p were predicted by

472   starBase.

473   D-F. The interaction between CROT and miR-33b-5p was validated via

474   luciferase reporter, RIP and RNA pull down assays.

475   G. The efficiency of miR-33b-5p inhibition was examined by qRT-PCR.

476   H. The mRNA and protein levels of CROT in different groups were

477   detected via qRT-PCR and western blot.

478   I. The expression of CROT in CRC cell lines and NCM460 cells were

479   tested by qRT-PCR.

480   J. The expression of CROT in SW620 and HT29 cells transfected with

481   sh-NC, sh-CROT#1 and sh-CROT#2 was detected by qRT-PCR.

482   K. The colony formation assay was done to test the effect of CROT

483   knockdown on cell proliferation.

484   L. TUNEL assay was implemented to examine the impact of silenced

485   CROT on cell apoptosis. Scale bar: 200 μm. $^{**}P < 0.01$.

486   **Figure 4 LncRNA EGOT regulated the proliferation and apoptosis of**

487   **CRC by targeting miR-33b-5p/CROT axis.**

488   A. The efficiency of CROT overexpression was detected by qRT-PCR in

489   SW620 and HT29 cells.

490   B. The proliferation of transfected cells was assessed via colony

491   formation assay.

492   C. TUNEL experiment was used to analyze the apoptosis of transfected

493   cells. Scale bar: 200 μm.

494   D. The expression of apoptosis-associated proteins was measured via

495   western blot. $^{*}P < 0.01$.

496   **Supplementary Table 1**

497   The sequences of primers were listed.

498   **Supplementary Table 2**

499   The shRNA sequences were shown.