Expression of miRNA in 5-FU resistant esophageal cancer

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Abstract. Fluoropyrimidine plus platinum (FP) are chemotherapeutic drugs that are most frequently used to treat esophageal squamous cell carcinoma (ESCC). However, drug resistance often occurs, and the mechanisms of resistance to 5-FU is yet to be determined. The role of micro (mi)RNAs has been well established in a variety of human cancers. The aim of the present study was to investigate the expression profile of ESCC, revealing the differential expression between ESCC and 5-FU resistant ESCC. The establishment of a 5-FU resistant (5-FUR) cell lines model provides a way of analyzing the expression of miRNAs in drug resistance. The miRNA expression indicated 50 miRNAs that were upregulated in TE10-5-FUR compared with TE10, while 119 miRNAs were downregulated. The TE11-5-FUR demonstrated 140 miRNAs were upregulated compared with TE11, which exhibited 12 downregulated miRNAs. Both cell lines share the 2 candidate upregulated miRNAs (miR-146a and miR-483-5p) and 5 downregulated miRNAs (miR-34a, miR-141, miR-200b, miR-200c and miR-205). Further studies are required to analyze and evaluate the function of the miRNAs.

Introduction

Cancer is a leading cause of human death. Esophageal cancer is the nine most common cancer worldwide, with an estimated 572,034 new cases and 508,585 deaths in 2018. Men have a substantially higher incidence than women (1). The cancers arise from the esophageal mucosa. There are two main histological types: Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC is the predominant histological type in southeast Asian countries, including Thailand (2). Major risk factors for ESCC are smoking and excessive alcohol consumption (3,4). These risk factors may lead to esophageal cancer through multiple genetic alterations, such as activated oncogenes and inhibited tumor suppressor genes (5).

While recent advances in surgical techniques and perioperative management, the prognosis of patients who undergo surgery alone for esophageal cancer remains poor. Neoadjuvant chemotherapy and chemoradiotherapy followed by surgery have emerged as a promising strategy for advanced esophageal cancer, and, in fact, good responders to such preoperative therapy show improved survival (6-9). Cisplatin/5-fluorouracil (5-FU) has been accepted as a standard treatment in for ESCC (10). However, following Cisplatin/5-FU based chemotherapy, non-responders are likely to receive no survival benefit (11,12). The ability to predict the response to chemotherapy before treatment should limit the application of chemotherapy to selected ESCC patients who are likely to show benefits. However, the prognosis of patients who are resistant to 5-FU treatment is poor. Resistance to treatment with anticancer drugs results from a variety of factors, including individual variations in patients.

miRNAs are noncoding RNAs that are approximately 22 nucleotides in length. They act through repressing the translation of target mRNAs by binding to the 3'-untranslated region of those mRNAs (13). miRNAs exist stably in various tissues and play pivotal roles in differentiation and development (14,15). The role of miRNAs has been well established in various human cancers. The evidence has shown that miRNA mutations or misexpression correlates with various human cancers, indicating that miRNAs can function as tumor suppressors or oncogenes. In addition, aberrant expression of miRNAs has been reported in various types of cancers (16,17). Recent studies of ESCC reported the oncogenic miRNAs: miR-21, miR-10b, miR-31, and miR-373; the oncosuppressor microRNAs: let-7, miR-34a, miR-133a, miR-150, miR-375, miR-205, miR-145, miR-29c, and miR-210 (18). The mi-R-25, mi-R-99a, mi-R-133a and mi-R-133b showed good potential as diagnostic markers and interestingly the mi-R-21, mi-R-27b, mi-R-126, mi-R-143 and mi-R-145 appeared to be useful both as diagnostic and prognostic/predictive markers (19). A recent publication showed the involvement of several miRNAs in resistance to 5-FU treatment as follows: The miRNA profiles of neoadjuvant radiochemotherapy non-responders showed upregulation of has-miR-1323, has-miR-3678-3p, hsv2-miR-H7-3p, has-miR-194, has-miR-3152, kshv-miR-K12-4-3p, has-miR-665 and has-miR-3659, and downregulation of has-miR-126, has-miR-484, has-miR-330-3p and has-miR-3653 (20). The aim of this study was to investigate
the expression profile of ESCC, revealing differential expression between ESCC and 5-FU resistant ESCC.

Materials and methods

Cell lines and cell culture. Human ESCC cell lines (TE4, TE10, TE11 and TE15) were obtained from Tohoku University. All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Nacalai Tesque, Inc.) containing 10% fetal bovine serum (Life Technologies Inc.), 10% penicillin/streptomycin (100 U/ml penicillin and 100 µg/ml streptomycin) (Nacalai Tesque, Inc.) in a humidified atmosphere under 5% CO₂ at 37°C.

Establishment of 5-FU resistant cell lines. 5-FU resistant (5-FUR) cell lines were cultured through gradual increases in 5-FU concentration. The cultured cells were exposed to 5-FU at an initial concentration of 1 nM/ml. After 24 h, the cells were cultured in 5-FU free medium until confluence. Next, 5-FU concentrations were increased by 2- to 3-fold and the cycle was repeated.

Proliferation assay. The WST-8 (2-(2-methoxy-4-nitrophyphenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) assay was conducted as described by the manufacturer (Nacalai Tesque Inc.) and was used to determine the IC₅₀ (50% growth inhibition concentration) value of 5-FU. Cells were plated in 96-well microplates and cultured for 12 h before exposure to various concentrations of 5-FU (0, 0.5, 1, 5, 10, 50 µg/ml) for 48 h. The optical density (OD) value was detected by RAINBO SUNRISE (Wako Pure Chemical Industries Ltd.) at 450 nm test wavelength and 650 nm reference wavelength. The IC₅₀ value of 5-FU was calculated from the dose-response curve.

Isolation of miRNA and miRNA microarray. The miRNA was isolated from the cell lines using the mirVana™ microRNA Isolation kit according to the manufacturer's protocol (Ambion; Thermo Fisher Scientific, Inc.). The concentration of RNA was quantified using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The miRNA expression profiling of human ESCC cell lines (TE10, TE11) and their corresponding 5-FU resistant (5-FU) daughter lines (TE10 5-FU resistant cells; TE10-5-FUR; TE11 5-FU resistant cell; TE11-5-FUR) were examined by TaqMan® Human MicroRNA Array (Applied Biosystems; Thermo Fisher Scientific, Inc.). It contained 384 miRNA targets (and 7 control miRNAs) and was performed using Megaplex™ RT Primers. The miRNA microarray analysis was performed with Applied Biosystems 7900HT fast real-time PCR System and RT-PCR StatMiner™ software. The expression of each miRNA in 5-FU resistant cell lines was compared with that in the control parental cell line, and the ratio of miRNA expression in 5-FU resistant cells to control cells was calculated for all 384 miRNAs.

Statistical analysis. The significance validation data of miRNA expression are expressed as mean ± standard error of the mean. The cell viability is computed and differences between viability curves are compared. The parameters are compared using the χ² test for categorical data and continuous variables are compared using Student's t-test. All data were analyzed with SPSS 22.0 data (IBM Corp.). A P-value <0.05 was considered to be statistically significant.

Results

The study first established 5-FU resistant ESCC cell lines (TE4, TE10, TE11 and TE15) by gradually increasing 5-FU concentration (starting from 0.1 µg/ml) and evaluating the cultures by WST-8 assay every 4 weeks. After 8 weeks, we determined the IC₅₀ values. The results showed a significant fold-increase in the concentration of 5-FU that inhibited TE10 and TE11 cell growth by 50%. TE10-5-FUR cells were relatively resistant to 5-FU, with an IC₅₀ of 42.66±2.38 µg compared to a value of 4.08±2.06 µg in the parent cells, a 10.5-fold increase in concentration (P<0.01). TE11-5-FUR cells were also relatively resistant to 5-FU with an IC₅₀ of 21.62±11.91 µg compared to 2.73±0.81 µg in TE11 parent cells, a 7.91-fold increase (P<0.01) (Fig. 1).

To assess miRNA expression levels, we used miRNA microarray to evaluate both 5-FU resistant and wild-type ESCC cell lines. The results of the miRNA expression study were subjected to a differential expression analysis and visualized using Expression Suite Software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The analysis showed 50 miRNAs upregulated in TE10-5-FUR compared to TE10, while 119 miRNAs were downregulated. The TE11-5-FUR demonstrated 140 miRNAs upregulated compared to TE11 with 12 miRNAs were downregulated. Among the most significantly upregulated miRNAs of TE10-5-FUR were has-miR-203-4373008, has-miR-429-4373160, has-miR-155-4373165, has-miR-125b-4373148, has-miR-140-5p-4373747, has-miR-146a-4373132, has-miR-155-4373549, has-miR-196b-4373526, has-miR-302b-4373871, has-miR-499a-4373207, has-miR-483-5p-4395449 while the most downregulated were has-miR-14a-4395168, has-miR-130a-4373145, has-miR-141-4373137, has-miR-152-4395170, has-miR-183-4395380, has-miR-200a-4373809, has-miR-200b-4395362, has-miR-200c-4395411, has-miR-205-4373093, has-miR-429-4373203 (Table I). For TE11-5-FUR, the prominent upregulated miRNAs were has-miR-let7b-4395446, has-miR-let7c-4373167, has-miR-10b-4395329, has-miR-22-4373079, has-miR-137-4373301, has-miR-146a-4373132, has-miR-296-5p-4373066, has-miR-499b-4381011, has-miR-483-5p-4395449, has-miR-522-4395524, while all those downregulated were has-miR-18a-4395168, has-miR-155-4373165, has-miR-141-4373137, has-miR-200b-4395362, has-miR-200c-4395411, has-miR-205-4373093, has-miR-331-5p-4395344, has-miR-429-4373203, has-miR-708-4395452 (Table II). The result in both cell lines observed at intersection of 2 miRNAs upregulated (miR-146a and miR-483-5p) and 5 miRNAs downregulated (miR-34a, miR-141, miR-200b, miR-200c and miR-205) (Fig. 2). Identification of potential target genes of miRNAs associated with 5-FU resistant ESCC cell lines was essential to investigate their biological functions. Candidate miRNAs of both cell lines were reviewed using the database of miRNA.org site (http://www.microrna.org) (21).
Table I. The list of differentially expressed microRNAs in TE10-5-FUR vs. TE10.

| miRNAs          | Fold-change | Regulation | microRNA family |
|-----------------|-------------|------------|-----------------|
| has-miR-99a-4373008 | 5.25707     | Up         | miR-99a         |
| has-miR-100-4373160 | 3.79643     | Up         | miR-100         |
| has-miR-125b-4373148 | 4.09845     | Up         | miR-125b        |
| has-miR-140-5p-437374 | 3.76036     | Up         | miR-140-5p      |
| has-miR-146a-4373132 | 5.46298     | Up         | miR-146a        |
| has-miR-155-4375459 | 4.67046     | Up         | miR-155         |
| has-miR-196b-4395326 | 6.09578     | Up         | miR-196b        |
| has-miR-302b-4378071 | 4.67347     | Up         | miR-302b        |
| has-miR-499a-4373207 | 5.78943     | Up         | miR-499a        |
| has-miR-483-5p-4395449 | 4.54962     | Up         | miR-483-5p      |
| has-miR-34a-4395168 | 3.08635     | Down       | miR-34a         |
| has-miR-130a-4373145 | 1.87463     | Down       | miR-130a        |
| has-miR-141-4373137 | 2.98572     | Down       | miR-141         |
| has-miR-152-4395170 | 0.56493     | Down       | miR-152         |
| has-miR-152-4395380 | 1.59869     | Down       | miR-183         |
| has-miR-200a-4378069 | 2.78942     | Down       | miR-200a        |
| has-miR-200b-4395362 | 0.89423     | Down       | miR-200b        |
| has-miR-200c-4395411 | 2.05483     | Down       | miR-200c        |
| has-miR-205-4373093 | 1.68473     | Down       | miR-205         |
| has-miR-429-4373203 | 1.87439     | Down       | miR-429         |

miR, microRNA.

Figure 1. Quantitation of 5-FU resistance of esophageal squamous cell lines. TE4, TE4-5-FUR, TE10, TE10-5-FUR, TE11, TE11-5-FUR, TE15 and TE15-5-FUR cells were seeded into 96-well microplates (5x10^3 per well) 12 h before treatment and were then exposed to different concentrations (0, 0.5, 1, 5, 10 and 50 µg) of 5-FU for 48 h. The percentage of cellular proliferation was evaluated with WST-8. The 5-FU resistant TE10-5-FUR cells showed resistance to 5-FU with an IC_{50} value of 42.66±2.38 µg and TE11-5-FUR achieved an IC_{50} value of 21.62±11.91 µg, both P<0.01 compared to their parental cells. For 5-FU resistant TE4-5-FUR cells and TE15-5-FUR, the results were not different to their parental cells. Data are presented as means ± SD and evaluated using Student's t-test. WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt; SD, standard deviation.
Discussion

Esophageal cancer is a major global health problem. Squamous cell carcinoma is the main histological type. Current management of ESCC depends on the stage of the disease and includes surgery, chemotherapy and radiation therapy. In multimodal treatment of esophageal cancer, chemotherapy has an important role in combination with radiation therapy and/or surgery. Fluoropyrimidine plus platinum (FP) are the chemotherapeutic drugs most frequently used to treat ESCC. This regimen has been reported to be effective, with improved overall survival (22,23). However, drug resistance
often occurs, and the mechanisms of resistance to 5-FU are still not clear.

The establishment of 5-FU resistant (5-FUR) cell lines model provides an approach to analyze the mechanism of drug resistance. The resistant cells were created from their parental lines by exposing them to gradually increasing 5-FU concentrations for 2 months. The TE10-5-FUR and TE11-5-FUR lines were partially resistant to 5-FU with IC₅₀ values of 42.66±2.38 µg and 21.62±11.91 µg.

miRNAs are short noncoding RNAs that regulate gene expression and play an important role in human cancers. They can also modulate the sensitivity and resistance to anticancer drugs. This study demonstrated 50 miRNAs upregulated and 119 miRNAs downregulated in TE10-5-FUR, 140 miRNAs upregulated and 12 miRNAs downregulated in TE11-5-FUR, compared to their wild type. The result in both of cell lines found 2 candidate miRNAs upregulated (miR-146a and miR-483-5p) and 5 miRNAs were downregulation (miR-34a, miR-141, miR-200b, miR-200c and miR-205). Recent studies also showed the involvement of several miRNAs in resistance to anticancer treatment and roles in esophageal cancer. miR-146a has been reported as possibly being associated with the cisplatin-base susceptibility to lung cancer by downregulating cyclin J (23,24) and as a potential therapeutic target for multidrug-resistant lung cancer by targeting DNA damage inducible transcript 3 (25). Polymorphism in miR-146a could be associated with the lymph node metastasis and prognosis of gastric cancer patients treated with oxaliplatin and fluoropyrimidines (26). miR-483-5p has been described upregulation with might be a tumor promoter of ESCC that correlated with TNM stage and survival (27). miR-483-5p could inhibit mitochondrial fission protein FIS1 with significant association with cisplatin sensitivity and with overall survival (28). miR-34a has shown significantly expressed reduction in ESCC tissues and exerted its anticancer function by suppressing PLCE1 (29). miR-34a has shown upregulation in cisplatin sensitivity for lung cancer treatment via p53/miR-34a/MYCN axis (30), mediates oxaliplatin resistance of colorectal cancer cells by inhibiting macroautophagy via the TGF-β/Smad4 pathway (31) and the patients with high levels of expression were found to benefit more from 5-FU based chemotherapy than patients with low levels of expression with the potential targets including CREB1, Bcl-2, Notch 1, Sirt1, and E2F3 (32). For miR-141, the overexpression could abolish the self-renewal ability and carcinogenicity of esophageal cancer stem-like cells and decrease cell invasion and migration by suppressing TM4SF1 (33). It enhanced the self-effected of 5-FU and suppressed the malignant biological behaviors of colorectal cancer by MAP4K4 signaling pathway (34).

miR-141 was significantly decreased and correlated with advanced TNM stage and lymph node metastasis with predicted possible target MACC1 in gastric cardia adenocarcinoma (35). miR-200b has been reported down-regulated in the multi-drug resistance of small cell lung cancer via ZEB2 (36). For miR-200c, the serum levels in advanced ESCC patients were significantly increased and associated with poor outcome of platinum-based chemotherapy (37). miR-200c also related to 5-FU chemotherapy with the potential targets PTEN and E-cadherin in colorectal cancer (38-40). miR-205 has been published as a tumor suppressor in adenocarcinoma and an oncogene in squamous cell carcinoma of esophagus through regulation of epithelial-mesenchymal transition (EMT) (41) and the Spl-mediated transcriptional activation of miR-205 promotes radioresistance through PTEN via PI3K/AKT pathway in ESCC (42). For TE11-5-FUR, the let-7b and let-7c demonstrated upregulation with related to the previous publication that reported the let7 play the role of oncosuppressor microRNAs (18).

This study acknowledges its own limitations-the study, the sample size is too small to make any reasonable conclusion. The cell viability and miRNA microarray experiments are not performed on non-cancer cell lines as a control. This study is not verified the function analysis of miRNAs that have related to resistance to 5-FU. Their function should be testing by gene transfer or knockdown with in vitro studies. Alternatively, the association of these miRNAs with clinical efficacy of chemotherapy be examining in a cohort of patients with esophageal cancer. The current study revealed differentially regulated miRNAs that are involved in 5-FU resistant ESCC. The identification of miRNA expression profiles and candidates in 5-FU resistant ESCC could provide a better understanding of the mechanisms involved in chemo-sensitivity or resistance. By predicting the response to chemotherapy, one could offer another treatment option for patients who would otherwise be resistant. Further study is needed to select the potential targets and explore the pathways that are upregulated or downregulated after induction of 5-FU therapy. Moreover, these findings suggest that it may be helpful to develop novel strategies for targeted therapies in ESCC patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

PM and PT contributed to the conception and design of the study. PM contributed to data collection, conduction of the study, performed the experiments, analysis and interpretation of the data. PM and PT reviewed the manuscript, designed the figures and tables. All the authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394–424, 2018.

2. Nun-Anan P and Vilachon RK: Late stage and grave prognosis of esophageal cancer in Thailand. Asian Pac J Cancer Prev 16: 1749–1754, 2015.

3. Morita M, Kumashiro R, Kubo N, Nakashima Y, Yoshida R, Yoshinaga K, Saieki H, Emi Y, Kakeji Y, Sakaguchi Y, et al: Alcohol drinking, cigarette smoking, and the development of squamous cell carcinoma of the esophagus: Epidemiology, clinical findings, and prevention. Int J Clin Oncol 15: 126-134, 2010.

4. Toh Y, Oki E, Ohgaki K, Sakamoto Y, Ito S, Egashira A, Saieki H, Kakeji Y, Morita M, Sakaguchi Y, et al: Alcohol drinking, cigarette smoking, and the development of squamous cell carcinoma of the esophagus: Molecular mechanisms of carcinogenesis. Int J Clin Oncol 15: 135-144, 2010.

5. Huang FL and Yu SJ: Esophageal cancer: Risk factors, genetic association, and treatment. Asian J Surg 41: 210-215, 2018.

6. Hsu PK, Chen HS, Huang CS, Liu CC, Hsieh CC, Hsu HS, Wu YC and Wu SC: Patterns of recurrence after oesophagectomy and postoperative chemotherapy vs surgery alone for oesophageal squamous cell carcinoma. Br J Surg 104: 90-97, 2017.

7. Passquali S, Yim G, Vohra RS, Mccollin S, Nyanhongo D, Marriot P, Geh JI and Griffiths EA: Survival after neoadjuvant and adjuvant treatments compared to surgery alone for resectable esophageal carcinoma: A network meta-analysis. Ann Surg 265: 481-491, 2017.

8. Ando N, Izuka T, Ide H, Ishida K, Shinoda M, Nishimaki T, Takayiwi W, Watanabe H, Isono K, Aoyama N, et al: Serum plus chemotherapy compared with surgery alone for localized squamous cell carcinoma of the thoracic esophagus: A Japan clinical oncology group study-JCOG9920. J Clin Oncol 21: 4592-4596, 2003.

9. Ando N, Kato H, Igaaki H, Shinoda M, Ozawa S, Shimizu H, Nakamura T, Yabusaki H, Aoyama N, Kurita A, et al: A randomized trial comparing postoperative adjuvant chemotherapy with cisplatin and 5-fluorouracil versus preoperative chemotherapy for localized advanced squamous cell carcinoma of the thoracic esophagus (JCOG9907). Ann Surg Oncol 19: 68-74, 2012.

10. Haisley KR, Hart KD, Nabavizadeh N, Bensch KG, Vaccaro GM, Thomas CR Jr, Schipper PH, Hunter JG and Dolan JP: Neoadjuvant chemoradiotherapy with concurrent cisplatin/5-fluorouracil is associated with increased pathologic complete response and improved survival compared to carboplatin/paclitaxel in patients with locally advanced esophageal cancer. Dis Esophagus 30: 1-7, 2017.

11. Rumiateo E, Cavallin F, Boldrin E, Cagol M, Alfieri R, Basso D, Castoro C, Ancona E, Amadori A, Ruol A and Sander C: The MiRNA-146a rs2910164 polymorphism and specific cancer susceptibility: An updated meta-analysis. Fam Cancer 17: 459-468, 2018.

12. Shi L, Xu Z, Wu G, Chen X, Huang Y, Wang J, and Li L: Up-regulation of miR-146a increases the sensitivity of non-small cell lung cancer to DDP by downregulating cyclin J. BMC Cancer 17: 138, 2017.

13. Tan W, Liao Y, Qiu Y, Liu H, Tan D, Wu T, Tang M, Zhang S and Wang H: miRNA 146a promotes chemotherapy resistance in lung cancer cells by targeting DNA damage inducible transcript 3 (CHOP). Cancer Lett 459: 4592-4596, 2017.

14. Liao YQ, Liao YL, Li J, Peng LX, Wan YY and Zhong R: Polymorphism in miR-146a associated with clinical characteristics and outcomes in gastric cancer patients treated with adjuvant oxaliplatin and fluoropyrimidines. Onco Targets Ther 8: 2627-2633, 2015.

15. Xue L, Nan J, Dong L, Zhang C, Li H, Na R, He H and Wang Y: Upregulated miR-483-5p expression as a prognostic biomarker for esophageal squamous cell carcinoma. Cancer Biomark 19: 193-197, 2017.

16. Fan S, Chen WX, Lv XB, Tang QL, Sun LJ, Liu BD, Zhong JL, Liu CY, Wang YY, Li QX, et al: miR-483-5p determines mitochondrial fission and cisplatin sensitivity in tongue squamous cell carcinoma by targeting FIS1. Cancer Lett 362: 183-191, 2015.

17. Cui XB, Peng H, Li RR, Mu QJ, Yang L, Li N, Liu CX, Hu JM, Li SG, Wei Y, et al: MicroRNA-34a functions as a tumor suppressor by directly targeting oncogenic PCLE1 in Kazakh esophageal squamous cell carcinoma. Oncotarget 8: 92454-92469, 2017.

18. Song C, Lu P, Sun G, Yang L, Wang Z and Wang D: miR-34a sensitizes lung cancer cells to cisplatin via p53/miR-34a/ MYCN axis. Biochem Biophys Res Commun 482: 22-27, 2017.

19. Sun C, Wang FJ, Zhang HG, Xu XZ, Jia RC, Yao L and Qiao PF: miR-34a mediates oxaliplatin resistance of colorectal cancer cells by inhibiting macroautophagy through transforming growth factor-β/ Smad4 pathway. World J Gastroenterol 23: 1816-1827, 2017.

20. Zhang Q, Wang J, Li N, Liu Z, Chen Z, Li Z, Lai Y, Shen L and Gao F: miR-34a increases the sensitivity of colorectal cancer cells to 5-fluorouracil in vitro and in vivo. Am J Cancer Res 8: 280-290, 2018.

21. Xue L, Yu X, Jiang X, Dang X, Mao L, Guo L, Fan J, Fan Q and Wang L and Lu SH: TM4SF1 promotes the self-renewal of esophageal cancer stem-like cells and is regulated by miR-141. Oncotarget 8: 19274-19284, 2017.

22. Wang F, Zhao L, Zhang J, Meng Z, Zhou C, Wang G, Li S, Xu Z, Niu W and Wang D: Chemotherapy-induced miR-141/ MAP4K4 signaling suppresses progression of colorectal cancer. Signal Transduct Target Ther 3: 16018, 2018.

23. Li S, Zhu J, Li J, S and Li B: MicroRNA-141 inhibits proliferation of gastric cardia adenocarcinoma by targeting MACC1. Arch Med Sci 14: 588-596, 2018.
36. Fang S, Zeng X, Zhu W, Tang R, Chao Y and Guo L: Zinc finger E-box-binding homeobox 2 (ZEB2) regulated by miR-200b contributes to multi-drug resistance of small cell lung cancer. Exp Mol Pathol 96: 438-444, 2014.

37. Yu H, Yuan B, Jiang L, Lin M, Sheng H, Huang J and Gao H: Serum miR-200c and clinical outcome of patients with advanced esophageal squamous cancer receiving platinum-based chemotherapy. Am J Transl Res 6: 71-77, 2013.

38. Heydari K, Saidijam M, Sharifi MR, Dermani FK, Soleimani Asl S, Shabah N and Najafi R: The effect of miR-200c inhibition on chemosensitivity (5-FluoroUracil) in colorectal cancer. Pathol Oncol Res 24: 145-151, 2018.

39. Hur K, Toyama Y, Takahashi M, Balague F, Nagasaka T, Koike J, Hemmi H, Koi M, Boland CR and Goel A: MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 62: 1315-1326, 2013.

40. Diaz T, Tejero R, Moreno I, Ferrer G, Cordeiro A, Artells R, Navarro A, Hernández R, Tapia G and Monzo M: Role of miR-200 family members in survival of colorectal cancer patients treated with fluoropyrimidines. J Surg Oncol 109: 676-683, 2014.

41. Hezova R, Kovarikova A, Srovnal J, Zemanova M, Harustiak T, Ehrmann J, Hajduch M, Sachlova M, Svoboda M and Slaby O: MiR-205 functions as a tumor suppressor in adenocarcinoma and an oncogene in squamous cell carcinoma of esophagus. Tumour Biol 37: 8007-8018, 2016.

42. Pan F, Mao H, Bu F, Tong X, Li J, Zhang S, Liu X, Wang L, Wu L, Chen R, et al: Sp1-mediated transcriptional activation of miR-205 promotes radioresistance in esophageal squamous cell carcinoma. Oncotarget 8: 5735-5752, 2017.