Abstract. Esophageal squamous cell carcinoma (ESCC) is a major histological type of esophageal cancer, identified as a leading cause of tumor-associated death worldwide. In addition, long non-coding RNA (lncRNA) BRAF-activated non-coding RNA (BANCR) expression is increased in the plasma of patients with ESCC, which can be reversed by tumor resection. Thus, the aim of the present study was to investigate the underlying mechanism of BANCR in ESCC progression. The relative mRNA expression of BANCR was determined via reverse transcription-quantitative PCR. The cell behaviors of Eca-109 cells were detected using Cell Counting Kit-8, colony formation, wound healing and Transwell chamber assays. Finally, the expression levels of proteins involved in the Raf/MEK/ERK signaling pathway and cell metastasis were analyzed with western blotting. The results revealed that lncRNA BANCR was highly expressed in ESCC cells compared with normal esophageal cells. BANCR overexpression enhanced proliferation, migration and invasion of ESCC cells, and BANCR silencing exerted opposite effects. Moreover, BANCR overexpression induced activation of the Raf/MEK/ERK signaling pathway in ESCC cells. Notably, U0126, a specific MEK inhibitor, decreased MEK and ERK expression, and blocked the promotive effects of BANCR overexpression on the proliferation, migration and invasion of ESCC cells. Overall, lncRNA BANCR facilitated the proliferation, migration and invasion of ESCC cells via the Raf/MEK/ERK signaling pathway. Thus, lncRNA BANCR may be a promising target for inhibiting ESCC growth and metastasis.

Introduction

Esophageal squamous cell carcinoma (ESCC) is defined as the major histological type of esophageal cancer (1,2) and as the seventh leading cause of tumor-associated death globally (3). ESCC is one of the most malignant and aggressive tumors worldwide, with ~410,000 deaths occurring annually (4). According to previous findings, the 3-year overall survival rate in certain areas of China was only ~30% after radiotherapy alone, whereas the recurrence rate was as high as 60-80% due to the existence of certain risk factors (5,6). Despite progress in diagnostic technologies and therapeutics, including chemoradiotherapy and surgery, most patients are diagnosed at the advanced stage and the prognosis of patients with ESCC remains unsatisfactory owing to recurrence and metastasis (7-9), with an overall 5-year survival rate <30% (10). Previous studies have reported that several important parameters, such as life style as well as environmental and genetic factors, contribute to ESCC tumorigenesis (11,12); however, the underlying mechanisms triggering ESCC progression have not been fully defined.

Long non-coding RNAs (lncRNAs) are a class of non-protein coding transcripts >200 nucleotides in length (13). lncRNAs serve a crucial role in a variety of processes in diseases, and their alterations are recognized as drivers or suppressors for cancer progression, including ESCC (14,15). Rapidly accumulating evidence has suggested that lncRNA BRAF-activated non-coding RNA (BANCR) has crucial roles in the progression of various malignancies, including colorectal cancer (16), papillary thyroid carcinoma (17) and endometrial cancer (18), via promoting proliferation, migration and invasion of cancer cells. Moreover, a previous study has suggested that BANCR is highly expressed in tumor tissues compared with in non-cancerous tissues of patients with ESCC and its expression level was positively associated with tumor differentiation, metastasis and tumor stage (1). Moreover, increased
BANCR expression was observed in the plasma of patients with ESCC, which was then decreased following tumor resection (5). Additionally, BANCR has been revealed to exert regulative effects on the migration and invasion of ESCC cells via the Wnt/β-catenin signaling pathway (19). BANCR expression was also negatively associated with the survival rate of patients with ESCC, suggesting BANCR as a novel tumor biomarker for the early detection of ESCC progression (5). It has been reported that BANCR mediates ESCC progression by regulating insulin-like growth factor 1 receptor expression via microRNA-338-3p (20). However, the underlying mechanism of BANCR in ESCC pathogenesis has not been fully understood. Thus, the aim of the present study was to further investigate the molecular mechanism of BANCR in ESCC progression.

The aim of the present study was to investigate the role of BANCR in the pathogenesis of ESCC, and study its potential molecular mechanism. Thus, ESCC cells were transfected with overexpression plasmid and short hairpin RNAs (shRNAs) to regulate BANCR expression in order to determine whether BANCR could regulate the proliferative, migratory and invasive capabilities of ESCC cells.

Materials and methods

Cell culture and transfection. ESCC cell lines, including Eca-109, KYSE30, KYSE150 and TE-1, together with the normal esophageal Ht-1A cell line, were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The cell lines were cultured in RPMI-1640 medium with 10% FBS (Gibco; Thermo Fisher Scientific, Inc., USA) at 37°C with 5% CO₂. The MEK inhibitor, U0126 (Selleck Chemicals), was diluted using DMSO to a working concentration of 20 μM. Eca-109 cells were treated with U0126 for 24 h at 37°C.

Eca-109 cells at the logarithmic growth phase were transfected with 100 nM BANCR short hairpin (sh)RNAs or plasmids using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h and incubated for another 24 h before subsequent experimentation, according to the manufacturer’s instructions. The shRNAs were obtained from Shanghai GenePharma Co., Ltd. The shRNA sequences were as follows: sh-BANCR-1 forward, 5'-GGACUCUCAAUGGCAAAACGTUTT-3' and reverse, 5'-AACGCUUUGCCAUUGAGUCCTT-3'; sh-BANCR-2 forward, 5'-GGAGUGGGCGACUAUGCAATT-3' and reverse, 5'-UUACGUUAUGCCACACUACTT-3'; and shRNA-NC (scrambled) forward, 5'-UUUCUGCAGCGUGACAGTTT-3' and reverse, 5'-ACGUGACACGUUCCGAGATT-3'. The full-length sequences of BANCR (NR_047671) were inserted into pcDNA3.1 vectors (Invitrogen; Thermo Fisher Scientific, Inc.) and then reverse transcribed using the PrimeScript RT kit (Takara Bio, Inc.) to synthesize the cDNA. The prepared reaction mixture was incubated at 37°C for 60 min after brief centrifugation (14,000 x g, 10 min), followed by incubation at 85°C for 5 min for RT. Relative expression levels were determined using quantitative primers and SYBR® Premix Ex Taq™ reagent (Takara Bio, Inc.). The thermocycling conditions were as follows: Pre-denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 15 sec, 60°C for 30 sec and 72°C for 30 sec. The results were calculated using the 2⁻ΔΔCq method (21). The primers used for qPCR were: BANCR forward, 5'-ACAGGACTCTCATGGCAACG-3' and reverse, 5'-ATGAAGAAAGCCTGTTGCAATG-3'; and GAPDH (internal reference) forward, 5'-GGTCCTCCTGACTTCAAC-3' and reverse, 5'-AGCCAAATTCTGTTGTCATAC-3'.

Cell viability assay. The survival rate of Eca-109 cells in each group was determined using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay. Briefly, Eca-109 cells (2x10⁵ cells/well) were seeded into 96-well plates and transfected with BANCR overexpression plasmids or sh-BANCR, as aforementioned. After incubation for 24, 48 or 72 h, the CCK-8 reagent (10 µl/well) was added to Eca-109 cells for another 2 h. Absorbance at 450 nm was measured using an enzyme-linked immunosorbent assay plate reader (Bio-Rad Laboratories, Inc.).

Colony formation assay. After transfection, Eca-109 cells (1x10⁵/well) were seeded into 6-well plates. The cells were cultured for 14 days at 37°C without disturbance during the period of incubation to form the cell clusters. Subsequently, cell colonies were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet solution for 30 min at room temperature before being imaged, and colonies with diameters >0.5 mm were imaged and counted using a digital camera (Nikon Corporation).

Wound healing assay. Eca-109 cells (2x10⁵ cells/well) were seeded in 6-well plates and transfected until cell confluency reached ~80-90% on the following day. A straight linear wound was gently induced using 200-µl sterile pipette tips, and the cells were cultured in RPMI-1640 culture medium with 1% FBS at 37°C for 24 h. Subsequently, the wound was observed and photographed under a fluorescence microscope (Olympus IX53; Olympus Corporation) at five random fields (magnification, x200). The average distance between cells was calculated relative to the control by using ImageJ software (version 1.48; National Institutes of Health) to determine the wound closure rate.

Transwell chamber assay. After transfection, Eca-109 cells (1x10⁵ cells/well) were resuspended in FBS-free RPMI-1640 medium and seeded into the upper chambers of Transwell plates precoated with Matrigel at 37°C for 4 h. The lower chamber was filled with 500 µl culture RPMI-1640 medium containing 10% FBS, and then maintained at 37°C in 5% CO₂ for 24 h. After incubation, the membrane was fixed with 4% paraformaldehyde for 30 min at 37°C. The remaining cells on the upper surface of the filter membrane were removed gently, while cells at the lower surface of the membrane were stained.
with 0.1% crystal violet for 30 min at room temperature. The cells were captured in five random fields with a fluorescence microscope (Olympus IX53; Olympus Corporation) at x200 magnification.

**Western blotting.** Proteins were extracted from transfected Eca-109 cells using RIPA lysis buffer (Takara Bio, Inc.) containing protease inhibitors according to the manufacturer's instructions, and then quantified using a BCA assay kit. Total proteins (20 µg/lane) were loaded and separated via SDS-PAGE on 10% gel, and then separated proteins were transferred onto PVDF membranes. After membranes were blocked with 5% skimmed milk for 2 h at room temperature, primary antibodies against the following proteins were used overnight at 4˚C: Matrix metalloproteinase 2 (MMP2; 1:1,000; cat. no. ab181286; Abcam), MMP9 (1:1,000; cat. no. ab76003; Abcam), phosphorylated (p)-c-Raf (1:1,000; cat. no. 9423; Cell Signaling Technology, Inc.), c-Raf (1:1,000; cat. no. 9422; Cell Signaling Technology, Inc.), p-MEK1/2 (1:1,000; cat. no. 9121; Cell Signaling Technology, Inc.), MEK1/2 (1:1,000; cat. no. 9122; Cell Signaling Technology, Inc.), p-ERK1/2 (1:500; cat. no. 9101; Cell Signaling Technology, Inc.), ERK1/2 (1:1,000; cat. no. 9102; Cell Signaling Technology, Inc.) and GAPDH (1:1,000; cat. no. 5174; Cell Signaling Technology, Inc.). After washing with PBS, the membranes were incubated with HRP-conjugated secondary antibodies (1:5,000; Jackson ImmunoResearch, Inc.) at room temperature for 2 h, and then visualized using ECL chemiluminescence (Pierce; Thermo Fisher Scientific, Inc.). After western blot analysis was performed to determine the expression levels of MMP2 and MMP9, two markers associated with tumor invasion and metastasis. As shown in Fig. 3E, BANCR overexpression led to a significant upregulation of MMP2 and MMP9 expression in Eca-109 cells. These data suggested that BANCR overexpression enhanced the proliferation, migration and invasion of ESCC cells. Additionally, wound healing and Transwell chamber assays were performed to detect the migratory and invasive abilities, respectively. The data revealed that the migratory and invasive capacities were significantly elevated in Eca-109 cells from the BANCR group compared with those of the control and vector groups (Fig. 3A-D). Furthermore, statistical analyses were performed using SPSS 19.0 (IBM Corp.), and data are presented as the mean ± SEM from three independent experiments. In contrast to BANCR overexpression, BANCR silencing resulted in a significant decrease in the migratory and invasive abilities (Fig. 5A-D). BANCR silencing significantly suppressed the viability and proliferative ability of Eca-109 cells (Fig. 2B and C), suggesting the promoting effect of BANCR overexpression on the proliferation of ESCC cells. Additionally, wound healing and Transwell chamber assays were performed to detect the migratory and invasive abilities, respectively. The data revealed that BANCR overexpression significantly enhanced the viability and proliferative ability, respectively. The findings indicated that BANCR overexpression significantly enhanced the viability and proliferative ability of Eca-109 cells (Fig. 2B and C), suggesting the promoting effect of BANCR overexpression on the proliferation of ESCC cells. Additionally, wound healing and Transwell chamber assays were performed to detect the migratory and invasive abilities, respectively. The data revealed that the migratory and invasive capacities were significantly elevated in Eca-109 cells from the BANCR group compared with those of the control and vector groups (Fig. 3A-D). Furthermore, western blot analysis was performed to determine the expression levels of MMP2 and MMP9, two markers associated with tumor invasion and metastasis. As shown in Fig. 3E, BANCR overexpression led to a significant upregulation of MMP2 and MMP9 expression in Eca-109 cells. These data suggested that BANCR overexpression enhanced the proliferation, migration and invasion of ESCC cells. 

**Role of BANCR in the proliferation and migration of ESCC cells.** To confirm the role of BANCR in the proliferation and migration of ESCC cells, the behaviors of Eca-109 cells were analyzed under the condition of BANCR silencing. sh-BANCR was established to downregulate BANCR expression. RT-qPCR demonstrated that a significantly lower BANCR expression was observed in cells from the sh-BANCR-2 and shRNA-BANCR-1 groups, with a lower BANCR expression in the sh-BANCR-2 group (Fig. 4A). Therefore, shRNA-BANCR-2 was employed for further study. In contrast to BANCR overexpression, BANCR silencing significantly suppressed the viability and proliferative ability of Eca-109 cells (Fig. 4B and C), as well as the migratory and invasive abilities (Fig. 5A-D). Additionally, BANCR-knockdown resulted in a significant decrease in MMP2 and MMP9 expression (Fig. 5E). These data indicated that BANCR silencing inhibited the proliferation, migration and invasion of ESCC cells.
investigate the underlying mechanism of BANCR in ESCC progression, the phosphorylation levels of c-Raf, MEK1/2 and ERK1/2 were detected using western blot analysis. The results revealed that BANCR overexpression significantly increased the levels of p-c-Raf, p-MEK1/2 and p-ERK1/2 (Fig. 6A), suggesting that the Raf/MEK/ERK signaling pathway may be involved in the role of BANCR in the proliferation, migration and invasion of ESCC cells.

**BANCR activates the proliferation, migration and invasion of ESCC cells via the Raf/MEK/ERK signaling pathway.** To further investigate the mechanism of BANCR/Raf/MEK/ERK signaling in ESCC progression, the specific MEK inhibitor (U0126) was employed. Western blot analysis revealed that U0126 treatment significantly attenuated the increased levels of p-MEK1/2 and p-ERK1/2 induced by BANCR overexpression (Fig. 6B). In addition, U0126 treatment significantly suppressed the viability and proliferative ability of Eca-109 cells transfected with BANCR (Fig. 7A and B). Furthermore, migration and invasion were significantly inhibited in the U0126+BANCR group compared with the BANCR group (Fig. 7C-F), as well as MMP-2 and MMP-9 expression (Fig. 7G). These data revealed that U0126 treatment reversed the effects of BANCR overexpression on proliferation, migration and invasion of ESCC cells.

**Discussion**

At present, a variety of lncRNAs have been identified as master regulators of gene expression, and their alterations drive or impede cancer progression (22). Tumorigenesis regulated by lncRNAs is a complicated and multi-stage process, involving complex cell signaling pathways. Rapidly accumulating evidence has suggested that lncRNAs serve an important role in oncogenesis, representing a strategy of lncRNAs as biomarkers for the diagnosis, prognosis and therapy of various
Figure 3. IncRNA BANCR overexpression promotes the migration and invasion of esophageal squamous cell carcinoma cells. (A and B) Migratory ability of Eca-109 cells transfected with or without BANCR overexpression plasmid was determined by wound healing assay (scale bar, 100 µm). (C and D) Invasive ability of Eca-109 cells transfected with or without BANCR overexpression plasmid was analyzed using Transwell chamber assay (scale bar, 100 µm). (E) Protein expression levels of MMP2 and MMP9 were detected via western blot analysis in Eca-109 cells transfected with or without BANCR overexpression plasmid. Data are presented as the mean ± SEM from three independent experiments. "P<0.01 and ""P<0.001 vs. vector group. lncRNA BANCR, long non-coding RNA BRAF-activated non-coding RNA; MMP, matrix metalloproteinase.

Figure 4. IncRNA BANCR downregulation decreases the viability and proliferative capability of esophageal squamous cell carcinoma cells. (A) Relative BANCR expression was quantified by reverse transcription-quantitative PCR in Eca-109 cells transfected with or without sh-BANCR. (B) Viability of Eca-109 cells was evaluated using the Cell Counting Kit-8 assay. (C) Cell proliferation was assessed by colony formation assay. Data are presented as the mean ± SEM from three independent experiments. "P<0.01 and """"P<0.001 vs. sh-NC group. IncRNA BANCR, long non-coding RNA BRAF-activated non-coding RNA; sh, short hairpin RNA; NC, negative control.
Figure 5. lncRNA BANCR silencing inhibits the migration and invasion of esophageal squamous cell carcinoma cells. (A and B) Migratory ability of Eca-109 cells transfected with or without sh-BANCR was determined by wound healing assay (scale bar, 100 µm). (C and D) Invasive ability of Eca-109 cells transfected with or without sh-BANCR was analyzed using Transwell chamber assay (scale bar, 100 µm). (E) Protein expression levels of MMP2 and MMP9 were detected via western blot analysis in Eca-109 cells transfected with or without sh-BANCR. Data are presented as the mean ± SEM from three independent experiments. ***P<0.001 vs. sh-NC group. lncRNA BANCR, long non-coding RNA BRAF-activated non-coding RNA; MMP, matrix metalloproteinase; sh, short hairpin RNA; NC, negative control.

Figure 6. lncRNA BANCR overexpression induces activation of the Raf/MEK/ERK signaling pathway in esophageal squamous cell carcinoma cells. Protein expression levels were evaluated by western blotting in Eca-109 cells (A) transfected with or without BANCR overexpression plasmid, and (B) treated with U0126. Data are presented as the mean ± SEM from three independent experiments. **P<0.001 vs. control group; #P<0.05 and ###P<0.001 vs. BANCR group. lncRNA BANCR, long non-coding RNA BRAF-activated non-coding RNA; p, phosphorylated; t, total.
types of cancer, including head and neck cancer and thyroid cancer (23,24). Recently, increasing evidence has shown that lncRNA BANCR may be an oncogene or a tumor suppressor in various human malignancies, including colorectal (16), pancreatic (25), non-small cell lung (26) and ovarian cancer (27). Notably, BANCR expression is significantly increased in tumor tissues of patients with ESCC compared with in non-cancerous tissues, and its level is positively associated with tumor differentiation, metastasis and tumor stage (1). However, the molecular mechanism of BANCR in the pathogenesis of ESCC remains unexploited. Thus, it is of great importance to investigate the underlying mechanism of BANC in ESCC progression.

Previously, BANCR expression has been observed to be upregulated in colorectal cancer tissues, and BANCR downregulation decreases cell proliferation and invasion, but activates apoptosis (16). Moreover, BANCR expression has been reported to be upregulated in retinoblastoma tissues and cell lines, and positively associated with tumor size and optic nerve invasion (28). Additionally, BANCR-knockdown significantly suppresses the proliferation, migration and invasion of retinoblastoma cells (28). In the present study, it was observed that BANC expression was higher in ESCC cell lines than in normal esophageal cells. In addition, BANCR overexpression enhanced the proliferation, migration and invasion of ESCC cells, while BANCR silencing exhibited opposite effects, which was consistent with a previous study (28). The current findings indicated that BANCR acted as an oncogene in ESCC progression.

Increasing evidence has revealed that the Raf/MEK/ERK signaling pathway is closely associated with tumorigenesis.
in multiple types of malignant tumor, including colon and lung cancer (29-31). A previous study has revealed that diallyl disulfide treatment elevates the apoptotic rate by downregulating the RAF/MEK/ERK signaling pathway in esophageal carcinoma cells (32). Additionally, BANCR promotes the migration and invasion of cancer cells and induces epithelial-mesenchymal transition in thyroid cancer through the Raf/MEK/ERK signaling pathway (17). In the present study, BANCOR overexpression induced activation of the Raf/MEK/ERK signaling pathway in ESCC cells, suggesting the participation of the Raf/MEK/ERK signaling pathway under BANCOR in ESCC progression. In the current study, U0126, a specific MEK inhibitor, decreased MEK and ERK expression, and blocked the promotive effects of BANCOR overexpression on the proliferation, migration and invasion of ESCC cells. Overall, the present results strongly suggested that IncRNA BANCOR accelerated proliferation, migration and invasion of ESCC cells via the Raf/MEK/ERK signaling pathway.

In conclusion, the current findings provided evidence to demonstrate that BANCOR expression was upregulated in ESCC cell lines. In addition, IncRNA BANCOR overexpression promoted the proliferation, migration and invasion of ESCC cells, which was significantly reversed following BANCOR downregulation. Thus, IncRNA BANCOR may be a promising target for inhibiting ESCC cell migration.

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
XY and GY designed the experiment and drafted the manuscript. XY, MH and GY performed the experiments and analyzed the data. GY reviewed the manuscript. XY and GY confirm the authenticity of all the raw data. All authors read and approved the final 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. Sadeghpour S and Ghobrian S: Evaluation of the potential clinical prognostic value of IncRNA-BANCOR gene in esophageal squamous cell carcinoma. Mol Biol Rep 46: 991-995, 2019.
2. Li X, Xiao X, Chang R and Zhang C: Comprehensive bioinformatics analysis identifies IncRNA HCG22 as a migration inhibitor in esophageal squamous cell carcinoma. J Cell Biochem 121: 468-481, 2020.
3. 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.
4. Shang QX, Yang YS, Yuan Y, Gu YM, Zhang HL, Ji AF and Chen LQ: Clinical and prognostic effects of adjuvant therapy on less advanced esophageal squamous cell carcinoma patients. Ann Biomed Med 9: 681-699, 2020.
5. Liu Z, Yang T, Xu Z and Cao X: Upregulation of the long non-coding RNA BANCOR correlates with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Biomed Pharmacother 82: 406-412, 2016.
6. Zhu Y, Yuan T, Zhang Y, Shi J, Bai L, Duan X, Tong R and Zhong L: AR-42: A pan-HDAC inhibitor with antitumor and antiangiogenic activities in esophageal squamous cell carcinoma. Drug Des Devel Ther 13: 4321-4330, 2019.
7. Shapiro J, van Lanschot JB, Huls Hof M, van Hagen P, van Berge Henegouwen M, Wijnhoven BP, van Laarhoven HW, Nieuwenhuijzen GAP, Hoppers GAP, Bonenkamp JJ, et al: Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): Long-term results of a randomised controlled trial. Lancet Oncol 16: 1090-1098, 2015.
8. Tachimori Y, Ozawa S, Namasu K, Ishihara R, Matusuba H, Muro K, Oyama T, Toh Y, Udagawa H and Uno T; Registration Committee for Esophageal Cancer of the Japan Esophageal Society: Comprehensive registry of esophageal cancer in Japan, 2011. Esophagus 15: 127-152, 2018.
9. van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MJ, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hoppers GA, Bonenkamp JJ, et al: Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 366: 2074-2084, 2012.
10. He LR, Liu MZ, Li BK, Jia WH, Zhang Y, Liao YJ, Chen YC, Zhang L, Guan XY, Zeng YX, et al: High expression of EZH2 is associated with tumor aggressiveness and poor prognosis in patients with esophageal squamous cell carcinoma treated with definitive chemoradiotherapy. Int J Cancer 127: 138-147, 2010.
11. Liu J, Wang W, Tang Y, Zhou D, Gao Y, Zhang Q, Zhou H, Xing L and Yu J: mRNA and methylation profiling of radioresistant esophageal cancer cells: The involvement of SALL2 in acquired aggressive phenotypes. J Cancer 8: 646-656, 2017.
12. Ghobrian S and Ardekani AM: Non-invasive detection of esophageal cancer using genetic changes in circulating cell-free DNA. Avicenna J Med Biotechnol 4: 3-13, 2012.
13. Liu J, Zhao SY, Jiang Q, Qu Y, Huang X, Du J, Sun W and Ye Q: Long noncoding RNA MYLK-AS1 promotes growth and invasion of hepatocellular carcinoma through the EGFR/HER2-ERK1/2 signaling pathway. Int J Biol Sci 16: 1989-2000, 2020.
14. Liu JQ, Deng M, Xue NN, Li TX, Guo YX, Gao L, Zhao D and Fan RT: IncRNA KLF3-AS1 suppresses cell migration and invasion in ESCC by impairing miR-185-5p-targeted KLF3 inhibition. Mol Ther Nucleic Acids 20: 231-241, 2020.
15. Wu D, He X, Wang W, Hu X, Wang K and Wang M: Long noncoding RNA SNHG12 induces proliferation, migration, epithelial-mesenchymal transition and stemness of esophageal squamous cell carcinoma cells via post-transcriptional regulation of BMI1 and CTNNB1. Mol Oncol 14: 2332-2351, 2020.
16. Ma S, Yang D, Liu Y, Wang Y, Lin T, Li Y, Yang S, Zhang W and Zhang R: LncRNA BANCOR promotes tumorigenesis and enhances adriamycin resistance in colorectal cancer. Aging (Albany NY) 10: 2062-2078, 2018.
17. Wang Y, Gu J, Lin X, Yan W, Wang W and Wu G: IncRNA BANCOR promotes EMT in PTC via the Raf/MEK/ERK signaling pathway. Oncol Lett 15: 5865-5870, 2018.
18. Wang D, Wang D, Wang N, Long Z and Ren X: Long non-coding RNA BANCOR promotes endometrial cancer cell proliferation and invasion by regulating MMP2 and MMP1 via ERK/MAPK signaling pathway. Cell Physiol Biochem 40: 644-656, 2016.
19. Chen Q, Zheng Y, Wu B, Chen X, Sun F, Ge P and Wang P: BANCR regulates the cell invasion and migration in esophageal squamous cell carcinoma through Wnt/beta-catenin signaling pathway. Onco Targets Ther 12: 9319-9327, 2019.

20. Song W, Wang K, Yang X, Dai W and Fan Z: Long non-coding RNA BANCR mediates esophageal squamous cell carcinoma progression by regulating the IGF1R/Raf/MEK/ERK pathway via miR-338-3p. Int J Mol Med 46: 1377-1388, 2020.

21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25: 402-408, 2001.

22. Peng WX, Koirala P and Mo YY: LncRNA-mediated regulation of cell signaling in cancer. Oncogene 36: 5661-5667, 2017.

23. Cossu AM, Mosca L, Zappavigna S, Misso G, Bocchetti M, De Micco F, Quagliuolo L, Porcelli M, Caraglia M and Boccellino M: Long non-coding RNAs as important biomarkers in laryngeal cancer and other head and neck tumours. Int J Mol Sci 20: 3444, 2019.

24. Mahmoudian-Sani MR, Jalali A, Jamshidi M, Moridi H, Alghasi A, Shojaeian A and Mobini GR: Long non-coding RNAs in thyroid cancer: Implications for pathogenesis, diagnosis, and therapy. Oncol Res Treat 42: 136-142, 2019.

25. Wu X, Xia T, Cao M, Zhang P, Shi G, Chen L, Zhang J, Yin J, Wu P, Cai B, et al: LncRNA BANCR promotes pancreatic cancer tumorigenesis via modulating MiR-195-5p/Wnt/beta-catenin signaling pathway. Technol Cancer Res Treat 18: 153303819889962, 2019.

26. Yang L and Liu G: LncRNA BANCR suppresses cell viability and invasion and promotes apoptosis in non-small-cell lung cancer cells in vitro and in vivo. Cancer Manag Res 11: 3565-3574, 2019.

27. Xu X, Pan L, Zhuo M, Yang X, Zhang W, Sun D, Zeng N and Zhang D: Increased expression of LncRNA BANCR and its prognostic significance in human epithelial ovarian cancer. Eur J Gynaecol Oncol 38: 449-452, 2017.

28. Su S, Gao J, Wang T, Wang J, Li H and Wang Z: Long non-coding RNA BANCR regulates growth and metastasis and is associated with poor prognosis in retinoblastoma. Tumour Biol 36: 7205-7211, 2015.

29. Roberts PJ and Der CJ: Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26: 3291-3310, 2007.

30. Wang Z, Ma L, Su M, Zhou Y, Mao K, Li C, Peng G, Zhou C, Shen B and Dou J: Baicalin induces cellular senescence in human colon cancer cells via upregulation of DEPP and the activation of Ras/Raf/MEK/ERK signaling. Cell Death Dis 9: 217, 2018.

31. Wu GJ, Pen J, Huang Y, An S, Liu Y, Yang Y, Hao Q, Guo XX and Xu TR: KAP1 inhibits the Raf-MEK-ERK pathway to promote tumorigenesis in A549 lung cancer cells. Mol Carcinog 57: 1396-1407, 2018.

32. Yin X, Zhang J, Li X, Liu D, Feng C, Liang R, Zhuang K, Cai C, Xue X, Jing F, et al: DADS suppresses human esophageal xenograft tumors through RAF/MEK/ERK and mitochondria-dependent pathways. Int J Mol Sci 15: 12422-12441, 2014.