LncRNA MALAT1 increases the stemness of gastric cancer cells via enhancing SOX2 mRNA stability
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Gastric cancer is one of the most common malignancies globally; cancer stem cells (CSCs) are regarded as being at the root of tumor progression, and there is thus a need to identify potential drugs to target CSCs. The long non-coding RNA MALAT1 promotes epithelial–mesenchymal transition and angiogenesis in colorectal cancer, but it is unknown whether it affects the stemness of gastric cancer cells. Here, we found that knockdown (KD) of MALAT1 attenuated the stemness of non-adherent gastric cancer cell spheroids, as evidenced by a decrease in primary and secondary spheroid formation capacity and expression of stemness markers. In contrast, overexpression (OE) of MALAT1 enhanced the stemness of adherent gastric cancer cells. Notably, KD of MALAT1 enhanced radiosensitivity and chemosensitivity of gastric cancer cell spheroids. We report that MALAT1 directly binds to sox2 mRNA (which encodes a critical master pluripotency factor), enhances the mRNA stability and increases its expression; KD of sox2 partially reversed the effect of MALAT1 OE on the stemness of gastric cancer cells. Importantly, expression of MALAT1 and sox2 exhibited a positive correlation in clinical samples. Therefore, our results indicate the existence of a novel MALAT1–sox2 axis which promotes the stemness of gastric cancer cells and may be a potential target for gastric cancer.

Gastric cancer is one of the most common malignancies in the world. The latest GLOBOCAN statistics estimates that there were about 1.033 million new cases of gastric cancer worldwide in 2018 (1/18 people), in which about 783 000 (1/12 people) of the patients died, and the disease rate and mortality rate rank the fifth and second, respectively [1]. Surgery is the most effective treatment, but recurrence often happens.

Cancer stem cells (CSCs) are regarded as being at the root of tumor progression, and many investigations have endeavored to discover potential drugs targeting CSCs. One previous study indicated that KU711 and WGA-TA, as novel heat shock protein 90 inhibitors, could target CSC function in differentiated and anaplastic thyroid cancers [2]; Abdollahpour-Alitappeh et al. developed specific monoclonal antibodies directed against CD123, a CSC marker of human acute myeloid leukemia [3]; Tanshinone IIA could inhibit cervical CSC migration and invasion by inhibiting YAP transcriptional activity [4]. However, up to now there are no effective drugs that can kill CSCs in clinical use, which might be due to undetermined mechanisms contributing to CSC progression.

Long non-coding RNAs (lncRNAs) are about 200 nt or 1 × 105 bp in length and lack an obvious open reading frame [5]. Recent studies have shown lncRNA roles in CSC progression. For example, lncRNA HAND2-AS1 could maintain the stemness of non-
small cell lung cancer cells [6]; a novel lncRNA, ZNF281, inhibits the self-renewal capacity of glioma stem-like cells via regulating the nuclear factor-κB1 signaling pathway [7]; and lncRNA UCA1 enhances the stemness of glioma cells by acting as a competitive endogenous RNA (ceRNA) for Slug [8]. LncRNA MALAT1 has been regarded as a druggable target in cancers [9]. It could induce the migration of human breast cancer cells [10]. MALAT1 promotes epithelial–mesenchymal transition (EMT) and angiogenesis by sponging miR-126-5p in colorectal cancer [11]. Additionally, MALAT1 exerts its oncogenic roles in osteosarcoma by regulating the miR-34a–cyclin D1 axis [12]. However, its effects on the stemness of gastric cancer cells remain unclear.

Herein, we show that MALAT1 positively regulated the stemness of gastric cancer cells and negatively regulated the radiosensitivity and chemosensitivity. We show that MALAT1 functioned as a post-transcriptional regulator of a critical pluripotency factor, sox2, further increasing the expression of another pluripotency factor, nanog. Importantly, sox2 is necessary for MALAT1-mediated effects on the stemness of gastric cancer cells. Notably, we firstly demonstrated the positive correlation between MALAT1 and sox2 expression in gastric cancer tissues. Collectively, our work reveals a novel MALAT1–sox2 regulatory axis which promotes the stemness of gastric cancer cells, providing insight into the regulation of gastric cancer cell stemness.

Materials and methods

Clinical samples, cell culture and reagents

Thirty pairs of gastric cancer and normal adjacent tissue samples were collected from the Changzhou Second People’s Hospital. Written informed consent from all patients and approval of the Hospital Ethic Review Committees were obtained. The study methodologies conformed to the standards set by the Declaration of Helsinki. Gastric cancer cell lines MKN-45 and SCG7901, and normal gastric epithelial cell GES-1 were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultured in 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) and antibiotics at 37 °C under a 5% humidified CO₂ atmosphere. After 10 days, the number and size of spheroid were evaluated and quantified under a microscope.

Spheroid formation analysis

Gastric cancer cells were cultured in ultra-low attachment 24-well plates (Corning, Union City, CA, USA) at 1000 cells/well with DMEM/F12 medium supplemented with 1 x B27 (Sigma Aldrich, St Louis, MO, USA), 20 ng·mL⁻¹ bFGF (MedChemExpress, Monmouth Junction, NJ, USA), 20 ng·mL⁻¹ EGF (MedChemExpress) and antibiotics at 37 °C under a 5% humidified CO₂ atmosphere. After 10 days, the number and size of spheroid were evaluated and quantified under a microscope.

Transwell migration assay

The detailed procedure is mentioned in a previous study [4]. Briefly, gastric cancer cells following different
treatments were digested and re-suspended, and 8 x 10⁴ cells were added to each upper chamber of a 24-well plate containing MILL1cell PET Hanging Cell Culture Inserts with a pore size of 8 μm PET (Millipore). Eight hundred microliters of medium containing 20% FBS was used as a chemo-attractant in the bottom chamber. After 24 h, cells migrating into the bottom chamber were fixed in methanol for 15 min and stained with 0.1% crystal violet for 15 min. Five random fields from each well were counted in triplicate by using phase contrast microscopy. Quantification was carried out with a microplate reader (attenuance at 570 nm) after destaining with 30% glacial acetic acid.

**Luciferase reporter assay**

The sequences of different regions of sox2 were cloned into pGL3-control plasmid (E1741; Promega, Madison, WI, USA), referred to as L3-UTR, L3-CDS, 3′UTR, which were co-transfected with β-gal into gastric cancer cells with Len-MALAT1-OE infection or not. After 72 h, cells were collected and luciferase activity was detected by ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay kit (E7110) (Promega) and VivoGlo Luciferin-β-gal Substrate kit (P1061) (Promega). β-Gal activity was used for normalization.

**mRNA stability assay**

MALAT1 was knocked down or overexpressed by lentivirus infection for 48 h. Then de novo RNA synthesis was blocked with adding 5 μg·mL⁻¹ of ActD (Apexbio, Madison, WI, USA) into the medium. Total RNA was harvested at indicated time points, and sox2 mRNA expression was detected by quantitative real-time PCR (qRT-PCR). The mRNA half-life of sox2 was determined by comparison with the mRNA level before adding ActD.

**RNA–RNA in vitro interaction assay**

The detailed procedure was described in previous work [13]. Briefly, 25 μL of Protein A/G MagneticBeads (Pierce, Rockford, IL, USA) was washed twice with RNA immunoprecipitation (RIP) wash buffer (Millipore; cat. no. CS203177) before incubating with BrU antibody (ab22848; Abcam, Cambridge, MA, USA) for 1 h at room temperature. After antibody conjugation, beads were washed twice with RIP wash buffer and then resuspended in incubation buffer containing RIP wash buffer, 17.5 mm EDTA (Millipore; cat. no. CS203175) and RNase Inhibitor (Millipore; cat. no. CS203219). Equal amounts (5 pmol) of BrU-labeled RNAs (MALAT1, MALAT1-Anti-sense, LacZ) were incubated with beads in Incubation Buffer for 2 h at 4 °C. Following incubation, 2.5 pmol of the sox2 5′UTR, CDS or 3′UTR RNA fragments was added into individual tubes and incubated overnight at 4 °C. After incubation, beads were digested, and RNA was extracted from the supernatant using the miRNeasy kit (Qiagen, Duesseldorf, Germany), and qRT-PCR was performed to detect sox2 5′UTR, CDS, 3′UTR levels.

**Cell viability analysis**

For the chemosensitivity assay, gastric cancer cells were cultured in 96-well plates at 3000 cells/well following by cisplatin (cat. no. HY-17394; MedChemExpress) treatment for 24, 48 and 72 h. For radiosensitivity, cells were treated with 3.2 Gy·min⁻¹ 6 MV X-ray vertical direct radiation using a Varian 2300EX Linear Accelerator (SPL Life Sciences, Gyeonggi-do, Korea), with source skin distance of 100 cm, for 24, 48 and 72 h. Cell viability was examined by using an Enhanced Cell Counting Kit-8 (cat. no. C0041; Beyotime) according to the manufacturer’s protocols.

**Statistical analysis**

All data are presented as the mean ± standard deviation (SD). Analysis of the datasets with only two groups was performed using Student’s t-test. The differences between the groups were analyzed using one-way ANOVA with the Tukey–Kramer post hoc test, and P < 0.05 was considered significant.

**Results**

**MALAT1 promotes the stemness of gastric cancer cells**

Since CSCs are regarded as being at the root of tumor progression, we wondered whether MALAT1 is involved in gastric cancer stemness. We chose SCG7901 and MKN45 cells as the research objects because MKN45 cells are poorly differentiated and SCG7901 cells are moderately differentiated; CSCs are poorly differentiated cells. Firstly, we found that MALAT1 OE significantly increased the expression of sox2 and nanog, well-established master pluripotency factors, while MALAT1 KD exerted the opposite effects (Fig. 1A–C). The KD and OE efficiency of MALAT1 in gastric cancer cells were confirmed by qRT-PCR analysis (Fig. 1D). Additionally, MALAT1 OE promoted the spheroid formation capacity of gastric cancer cells, as evidenced by increased spheroid size and number, whereas MALAT1 KD significantly decreased the capacity (Fig. 1E,F). As the ability of cells to form secondary tumor spheroids after dissociation and dilution to single cells is a measure of self-renewal capability, and thus serves as a surrogate CSC assay [14], we determined MALAT1’s effect on the ability of secondary spheroid formation. As expected,
secondary spheroid formation ability was remarkably inhibited by MALAT1 KD, while MALAT1 OE significantly facilitated it (Fig. 2A,B). Notably, we found that MALAT1 OE could endow GES-1 cells with stemness, characterized as the formation of spheroids, which was reversed by MALAT1 KD (Fig. 2C). These results suggest that MALAT1 promotes the stemness of gastric cancer cells.

MALAT1 increases the migration ability and epithelial–mesenchymal transition process of gastric cancer cells

As CSCs facilitate the migration and EMT process of tumor cells, we further investigated MALAT1-mediated effects on the migration and EMT process of gastric cancer cells. We found that MALAT1 OE increased the migration ability of gastric cancer cells, while MALAT1 KD suppressed the migration ability (Fig. 3A,B). Furthermore, MALAT1 OE increased the expression of a mesenchymal marker (vimentin) and decreased the expression of an epithelial marker (E-cadherin), while MALAT1 KD exerted the opposite effects (Fig. 3C–E).

MALAT1 KD enhances the chemo- and radiosensitivity of gastric cancer cells

We further explored the effects of MALAT1 on the chemo- and radiosensitivity of gastric cancer cells because CSCs have been confirmed to be resistant to chemo- and radiotherapy. As shown in Fig. 4A,B, MALAT1 KD enhanced the chemosensitivity and radiosensitivity of gastric cancer cells. In contrast, MALAT1 OE attenuated the chemosensitivity and radiosensitivity of gastric cancer cells (Fig. 4C,D).

Fig. 1. LncRNA MALAT1 promotes the stemness of gastric cancer cells. (A,B) The mRNA levels of sox2 and nanog were detected in gastric cancer cells with ectopic expression of MALAT1 by qRT-PCR assay. (C) The protein levels of sox2 and nanog were examined in gastric cancer cells with ectopic expression of MALAT1 by western blot assay. (D) The KD and OE efficiency of Len-MALAT1-KD and Len-MALAT1-OE were confirmed in gastric cancer cells by qRT-PCR analysis. (E,F) The capacity of spheroid formation was measured in the cells described in (A) by detecting spheroid size (E) and number (F). Scale bar, 100 μm. The difference was assessed using one-way ANOVA with the Tukey–Kramer post hoc test. Data are presented as the mean ± SD, n ≥ 3, **P < 0.01 vs. control.
Fig. 2. LncRNA MALAT1 enhances the secondary spheroid formation ability of gastric cancer cells. (A,B) The secondary spheroid formation capacity was evaluated in gastric cancer cells with ectopic expression MALAT1. (C) The spheroid formation ability was determined in GES-1 cells with or without MALAT1 OE. The difference was assessed using one-way ANOVA with the Tukey–Kramer post hoc test. Data are presented as the mean ± SD, n ≥ 3, **P < 0.01 vs. control. Scale bar, 100 μm.

Fig. 3. LncRNA MALAT1 increases the migration ability of gastric cancer cells. (A,B) The migration ability of gastric cancer cells with ectopic expression of MALAT1 was examined (A) and quantified (B). Scale bar, 200 μm. (C–E) The expression of EMT markers (E-cadherin and vimentin) was detected in the cells described in (A). The difference was assessed using one-way ANOVA with the Tukey–Kramer post hoc test. Data are presented as the mean ± SD, n ≥ 3, **P < 0.01 vs. control.
MALAT1 regulates the master stemness factors sox2 and nanog via directly binding to sox2 mRNA

Then, we explored the mechanisms by which MALAT1 exerts its effects on the stemness of gastric cancer cells. As lncRNAs have been shown to act as mRNA stabilizers [15–17], we wondered whether MALAT1 could bind to and stabilize sox2 mRNA. As expected, the decay rate of sox2 was faster in Len-MALAT1-KD-infected cells (t1/2 = 2.85 ± 0.3 h vs. 3.96 ± 0.2 h in SCG87901 cells; t1/2 = 2.76 ± 0.2 h vs. 3.3 ± 0.4 h in MKN-45 cells) (Fig. 5A,B), whereas the decay rate of sox2 was slower in Len-MALAT1-OE-infected cells. Furthermore, an in vitro RNA–RNA interaction assay indicated that MALAT1 directly bound to sox2 3’UTR in MKN-45 and SCG7901 cells, but not its 5’UTR or CDS (Fig. 5C, D). Consistently, luciferase reporter analysis showed that MALAT1 OE increased the activity of L3-sox2-3’UTR, while the activity of L3-sox2-5’UTR and L3-sox2-CDS was unaffected (Fig. 5E,F). Thus, our results show that MALAT1 may directly bind to sox2 mRNA to mediate alterations in gastric cancer cell stemness.

Fig. 4. Knockdown of MALAT1 enhances chemo- and radiosensitivity of gastric cancer cells. (A,B) The cell viability was measured in gastric cancer cells with ectopic expression of MALAT1 plus cisplatin treatment or not. (C,D) Gastric cancer cells with ectopic expression of MALAT1 were treated with 3.2 Gy-min⁻¹ followed by detecting the cell viability. The difference was assessed using one-way ANOVA with the Tukey–Kramer post hoc test. Data are presented as the mean ± SD, *P < 0.05, **P < 0.01 vs. control.
Fig. 5. MALAT1 regulates the master stemness factors sox2 and nanog via directly binding to sox2 mRNA. (A,B) The mRNA stability of sox2 was measured in gastric cancer cells with ectopic expression of MALAT1. (C,D) MALAT1 interacted with the sox2 3' UTR in an in vitro RNA–RNA interaction assay. Compared to a panel of control RNAs (MALAT1 antisense, control, MALAT1), MALAT1 binds to sox2 3' UTR with higher affinity. The binding affinity was quantified by qRT-PCR analysis of the sox2 3' UTR. Data were normalized to the MALAT1 control. (E,F) The luciferase activity of pGL3-control plasmid containing different regions of sox2 was determined in gastric cancer cells with ectopic expression of MALAT1. The difference was assessed using one-way ANOVA with the Tukey-Kramer post hoc test. Data are presented as the mean ± SD, n ≥ 3, **P < 0.01 vs. control.
Sox2 mediates the cancer stem cell-associated phenotypes downstream of MALAT1.

Then, we determined whether MALAT1-mediated effects on the stemness of gastric cancer cells were indeed through Sox2. As shown in Fig. 6A, B, the decreased expression resulting from MALAT1 KD was rescued by Sox2 OE. Additionally, the decreased capacity of spheroid formation induced by MALAT1 KD was partially abrogated by Sox2 OE (Fig. 6C, D). Furthermore, MALAT1 KD-mediated decrease of gastric cancer cell migration ability was attenuated by Sox2 OE (Fig. 6E, F). Moreover, the inhibitory effects on the EMT process were rescued by Sox2 OE (Fig. 6G). Finally, we performed correlation analysis...
of MALAT1 and sox2 mRNA expression in clinical samples. To our surprise, the expression of MALAT1 and sox2 displayed a positive correlation in gastric cancer tissues (Fig. 6H). As a result, this work indicates that the MALAT1–sox2 axis promotes the stemness of gastric cancer cells.

Discussion
This work demonstrated that lncRNA MALAT1 positively regulated the stemness of gastric cancer cells by directly binding to sox2 mRNA, enhancing sox2 mRNA stability. Although the promoting roles of MALAT1 have been established in other tumors, to our knowledge, this is the first work revealing MALAT1’s roles in gastric cancer cell stemness.

LncRNAs were initially regarded as 'junk genes', but a growing body of evidence suggests critical roles for them in tumor progression [9,18]. Although many studies have identified the oncogenic roles of lncRNA MALAT1 [11,12], some other studies showed that MALAT1 held tumor-suppressive roles. For example, Kwok et al. [19] characterized a non-canonical phosphatase and tensin homologue (PTEN)–microRNA–MALAT1 axis and first found that MALAT1 possesses novel tumor suppressive properties in colon and breast cancers. Additionally, MALAT1 suppresses glioma progression by decreasing miR-155 expression and activating FBXW7 function [20]. Furthermore, MALAT1 could inhibit glioma cell proliferation and metastasis by downregulating matrix metalloproteinase 2 and inactivating extracellular signal-regulated kinase/mitogen-activated protein kinase signaling [21]. These studies suggest that MALAT1 holds different functions in different tumors. In the present study, we firstly characterized the oncogenic role of MALAT1 in gastric cancer cell stemness. Notably, as previous studies have shown that sox2 regulates stemness upstream of nanog [22], this is consistent with our results showing that MALAT1 regulates nanog expression through sox2.

As critical epigenetic modulators, lncRNAs could exert their effects through various pathways; for example, lncRNAs could act as a co-activator or co-repressor for protein [23], mRNA [16] and DNA [24]. Additionally, recent research has demonstrated that lncRNAs could serve as miRNA sponges to repress miRNA activity [8,12], and moreover, lncRNA could affect gene location by regulating the variable splicing of genes [25,26]. LncRNA MALAT1 could exert its functions through different mechanisms in different conditions; for example, previous studies have indicated that MALAT1 could act as a ceRNA to regulate miRNA activity in breast cancer [10], osteosarcoma [12] and gastric cancer [27]. What is more, MALAT1 promotes hepatocellular carcinoma progression by binding BRG1 to epigenetically enhance inflammatory response in hepatocellular carcinoma tissues [28]. MALAT1 lncRNA binds to and inactivates the prometastatic transcription factor TEAD and thus suppresses breast cancer metastasis [29]. Here, we found that MALAT1 directly binds to the stemness master factor sox2 mRNA, increases sox2 mRNA stability and enhances the stemness of gastric cancer cells. However, we must admit that further in vivo experiments should be performed to confirm this conclusion.

Long non-coding RNA MALAT1 has been regarded as a potential therapeutic target in some kinds of tumors [30] and researchers have designed an antisense oligonucleotide-conjugated nanostructure targeting MALAT1 to inhibit cancer metastasis [31]. This work reveals the oncogenic role of the MALAT1–sox2 axis in gastric cancer cell stemness, which suggests that MALAT1 might be a potential target for gastric cancer treatment as well.

Conflict of interest
The authors declare no conflict of interest.

Author contributions
GW and YX conceived and designed the project, YX, JP and QG acquired the data, YX, JP and QG analyzed and interpreted the data, and GW and YX wrote the paper.

References
1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68, 394–424.
2 White PT, Subramanian C, Zhu Q, Zhang H, Zhao H, Gallagher R, Timmermann BN, Blagg BS and Cohen MS (2016) Novel HSP90 inhibitors effectively target functions of thyroid cancer stem cell preventing migration and invasion. Surgery 159, 142–151.
3 Abdollahpour-Alitappeh M, Razavi-Vakhshourpour S and Abolhassani M (2018) Development of a new anti-CD123 monoclonal antibody to target the human CD123 antigen as an acute myeloid leukemia cancer stem cell biomarker. Biotechnol Appl Biochem 65, 841–847.
4 Qin J, Shi H, Xu Y, Zhao F and Wang Q (2018) Tanshinone IIA inhibits cervix carcinoma stem cells
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14 Weiswald LB, Bellet D and Dangles-Marie V (2015) LncRNA MALAT1 increases the stemness of gastric cancer cells via enhancing SOX9 mRNA stability. *Biomed Pharmacother* **105**, 758–765.

5 Zhao L, Han T, Li Y, Sun J, Zhang S, Liu Y, Shan B, Zheng D and Shi J (2017) The lncRNA SNHG5/miR-32 axis regulates gastric cancer cell proliferation and migration by targeting KLF4. *FASEB J* **31**, 893–903.

6 Miao F, Chen J, Shi M, Song Y, Chen Z and Pang L (2019) LncRNA HAND2-AS1 inhibits non-small cell lung cancer migration, invasion and maintains cell stemness through the interactions with TGF-beta1. *Biosci Rep* **39**. https://doi.org/10.1042/BSR20181525.

7 Li XT, Li JC, Feng M, Zhou YX and Du ZW (2018) Novel lncRNA-ZNF281 regulates cell growth, stemness and invasion of glioma stem-like U251s cells. *Neoplasma* **66**, 118–127.

8 Li Z, Liu H, Zhong Q, Wu J and Tang Z (2018) LncRNA UCA1 is necessary for TGF-beta-induced epithelial-mesenchymal transition and stemness via acting as a ceRNA for Slug in glioma cells. *FEBS Open Bio* **8**, 1855–1865.

9 Amadio N, Raimondi L, Juli G, Stamato MA, Caracciolo D, Tagliaferri P and Tassone P (2018) MALAT1: a druggable long non-coding RNA for targeted anti-cancer approaches. *J Hematol Oncol* **11**, 63.

10 Chou J, Wang B, Zheng T, Li X, Zheng L, Hu J, Zhang Y, Xing Y and Xi T (2016) MALAT1 induced migration and invasion of human breast cancer cells by competitively binding miR-1 with cdc42. *Biochem Biophys Res Commun* **472**, 262–269.

11 Sun Z, Ou C, Liu J, Chen C, Zhou Q, Yang S, Li G, Wang G, Song J, Li Z et al. (2018) YAP1-induced MALAT1 promotes epithelial-mesenchymal transition and angiogenesis by sponging miR-126-5p in colorectal cancer. *Oncogene* **38**, 2627–2644.

12 Duan G, Zhang C, Xu C, Xu C, Zhang L and Zhang Y (2019) Knockdown of MALAT1 inhibits osteosarcoma progression via regulating the miR34a/cyclin D1 axis. *Int J Oncol* **54**, 17–28.

13 Zhang Y, Pitchiya S, Cieslik M, Niknafs YS, Tien JC, Hosono Y, Iyer MK, Yazdani S, Subramaniam S, Shukla SK et al. (2018) Analysis of the androgen receptor-regulated lncRNA landscape identifies a role for ARLN1C in prostate cancer progression. *Nat Genet* **50**, 814–824.

14 Weiswald LB, Bellet D and Dangles-Marie V (2015) Spherical cancer models in tumor biology. *Neoplasia* **17**, 1–15.

15 Jadaliha M, Gholamalamdari O, Tang W, Zhang Y, Petracovici A, Hao Q, Tariq A, Kim TG, Holton SE, Singh DK et al. (2018) A natural antisense lncRNA controls breast cancer progression by promoting tumor suppressor gene mRNA stability. *PLoS Genet* **14**, e1007802.
regulating the microRNA30b/autophagy related gene 5 axis in gastric cancer. *Int J Oncol* **54**, 239–248.

28 Huang M, Wang H, Hu X and Cao X (2019) IncRNA MALAT1 binds chromatin remodeling subunit BRG1 to epigenetically promote inflammation-related hepatocellular carcinoma progression. *Oncoimmunology* **8**, e1518628.

29 Kim J, Piao HL, Kim BJ, Yao F, Han Z, Wang Y, Xiao Z, Siverly AN, Lawhon SE, Ton BN *et al*. (2018) Long noncoding RNA MALAT1 suppresses breast cancer metastasis. *Nat Genet* **50**, 1705–1715.

30 Li ZX, Zhu QN, Zhang HB, Hu Y, Wang G and Zhu YS (2018) MALAT1: a potential biomarker in cancer. *Cancer Manag Res* **10**, 6757–6768.

31 Gong N, Teng X, Li J and Liang XJ (2018) Antisense oligonucleotide-conjugated nanostructure-targeting IncRNA MALAT1 inhibits cancer metastasis. *ACS Appl Mater Interfaces* **11**, 37–42.