NANOG Promotes Cell Proliferation, Invasion, and Stemness via IL-6/STAT3 Signaling in Esophageal Squamous Carcinoma

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Abstract
Background: Cancer cells have properties similar to those of stem cells, including high proliferation and self-renewal ability. NANOG is the key regulatory gene that maintains the self-renewal and pluripotency characteristics of embryonic stem cells. We previously reported that knockdown of the pluripotent stem cell factor NANOG obviously reduced the proliferation and drug-resistance capabilities of esophageal squamous cell carcinoma (ESCC). In this study, we gained insights into the potential regulatory mechanism of NANOG, particularly in ESCC. Methods: NANOG was ectopically expressed in the Eca-109 cell line via pcDNA3.1 vector transfection. The mRNA expression of different genes was detected using quantitative real-time polymerase chain reaction, and protein quantification was performed by western blotting. The enzyme-linked immunosorbent assay was used to detect the expression of interleukin 6 (IL-6). The capabilities of proliferation, migration, and invasion were investigated using cell count and Transwell assays. The tumor sphere-forming assay was used to investigate the sphere formation capacity of cancer stem cells. Results: The expression of NANOG promoted the cell proliferation and sphere formation capacity of cancer stem cells in a dose-dependent manner. IL-6-mediated activation of signal transducer and activator of transcription 3 (STAT3) was closely related to the expression of NANOG in ESCC. Consistently, the target genes of STAT3, including CCL5, VEGFA, CCND1, and Bcl-xL, were upregulated upon the overexpression of NANOG. Conclusion: These results revealed that the expression of NANOG promotes cell proliferation, invasion, and stemness via IL-6/STAT3 signaling in ESCC.

Keywords
esophageal cancer, NANOG, cell proliferation, IL-6, STAT3

Abbreviations
ESCs, embryonic stem cells; CSCs, cancer stem cells; ESCC, esophageal squamous cell carcinoma; ON cells, NANOG overexpression cells; ABCG2, ATP binding cassette subfamily G member 2; IL-6, interleukin 6; JAKs, Janus kinases; p-STAT3, phospho-STAT3; STAT3, signal transducer and activator of transcription 3; qPCR, quantitative real-time PCR

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Introduction
Esophageal cancer is the seventh most common cancer and the fourth leading cause of cancer-related deaths, with 5-year survival rates as low as 13%.1 Esophageal squamous cell carcinoma (ESCC) is the main type of esophageal cancer in Asia, ranking fourth by incidence in China.2 Currently, the treatment of esophageal cancer is mainly based on esophagectomy combined with radio- and chemotherapy; some natural products such as curcumin are expected to be used in adjuvant therapy.

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treatment. Therefore, it is vital to elucidate the molecular mechanisms of ESCC development, with the goal of early detection and therapy to inhibit further tumor progression.

Cancer cells often share many key biological properties with embryonic stem cells (ESCs), including tumor formation and self-renewal ability. These properties are often attributed to the expression of pluripotency genes, such as those of NANOG (Nanog homeobox), OCT3/4 (POU class 5 homeobox 1), and SOX2 (SRY-box transcription factor 2), which are essential transcription factors for maintaining ESC totipotency. Numerous studies have shown that many pluripotency factors are expressed in solid tumors and participate in tumor development. NANOG is one such mediator that is expressed in various cancers, such as ovarian, breast, and prostate cancers, and is enriched in cancer stem cells (CSCs).

Previously, we investigated the correlation between the expression of NANOG and the malignant characteristics of ESCC. We found that NANOG mRNA and protein were highly expressed in ESCC cell lines. Further, mRNA silencing technology was used to knock down NANOG expression in ESCC. We found that the clonal formation, proliferation, and drug resistance of Eca-109 cells decreased upon the downregulation of NANOG expression. Moreover, NANOG deficiency downregulated the expression of ATP binding cassette subfamily G member 2. Therefore, in this study, we mainly focused on the tumorigenic promoting effect and related mechanism of NANOG in ESCC.

Materials and Methods

Cell Culture and Transfection

Eca-109, KYSE-150, and TE-1 cells were purchased from the Shanghai cell bank, Chinese Academy of Sciences. The complete medium (DMEM with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin [Hyclone]) configuration was used for cell culture. shRNA against human NANOG have been described by Deng et al. Ectopic expression plasmids (control and NANOG) were constructed by Shanghai GeneChem Co., Ltd Lipofectamine 2000 (Invitrogen) was used for cell transfection. After 16 to 24 h, a selective medium (supplemented with 2.0 µg/mL puromycin) was used for cell culture. shRNA against human NANOG was used for cell culture. The ECL-Plus detection system (Bio-Rad) was used to visualize the bands.

Protein Extraction and Western Blot Analysis

The spheroid or adherent Eca-109 cells were collected and lysed in cell lysis buffer. Then, the lysates were vortexed and 4°C for 10 min. The protein samples were separated using 10% to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis for western blot analysis. The primary antibodies used were anti-NANOG (Santa Cruz, sc-134218, 1:2000), signal transducer and activator of transcription 3 (STAT3; HuaBio, ET1607 to 38, 1:1000), phospho-STAT3 (p-STAT3; HuaBio, ET1603 to 40, 1:1000), IL-6 (HuaBio, EM1701 to 45, 1:1000), phospho-Janus kinase 2 (p-JAK2; HuaBio, ET1607 to 34, 1:500), glyceraldehyde 3-phosphate dehydrogenase (Abcam, ab8245, 1:10000), PTEN (HuaBio, RT1519, 1:1000), and Phospho-PTEN(S380) (HuaBio, ET1701 to 46, 1:500). The HRP-conjugated anti-rabbit/mouse secondary antibody was used to enable detection. The ECL-Plus detection system (Bio-Rad) was used to visualize the bands.

Statistical Analysis

Results were expressed as means ± SD. The 2-tailed Student’s t-test was used to compare means between groups. P-values less than .05 were considered statistically significant.
Table 1. Primers for qPCR analysis.

| Genes  | Forward (5'-3')          | Reverse (5'-3')          |
|--------|--------------------------|--------------------------|
| NANOG  | ACCTATGCTGTGTAGTTTGTG    | AGTGGGTTGTGGTTCCTTTTG    |
| GAPDH  | ACATCGCTGACACACCATG      | TGTAGTGGAGGTCAATGAGGA    |
| Bcl-xl | GACATCCCAGCTCCACAATC     | GTTCCCCATAGGGTTCCACAAAGG |
| CCND1  | CATCTACACCGACAATCCATC   | TCTGCCATTTTTGAGGAAGAG    |
| c-Myc  | TTCCGGGTAGTGGAAAAACAG    | AGTAAAGATACGCTGACC       |
| MCL1   | AAGGACAAAGGGGACTGCG      | ATATGCCAAACAGCTCCTAC     |
| VEGFA  | AGTCCAAACATACCATGACG     | TTTCCCTCTTCGACTGATT      |
| Snail  | ACAAGCACCAAGAGCTCGG      | ATGGCAGTGAGAGAGAGTGG     |
| MMP9   | ACGTGAAACATCTTGCACCGCATC| TCAGAGAATCGCCAGTACTTCCC  |
| CCL5   | TGCCCATCAGGAGTATTTTC     | CCATCGTGCATCCTCACAAGG    |
| IL-6   | CCACCTACCTCTTCCAGAACG    | CATCTTTGAGGTTTCTCGTGTTG |

Abbreviations: qPCR, quantitative real-time; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Bcl-xl, B-cell lymphoma-extra-large; VEGFA, vascular endothelial growth factor A; CCND1, cyclin D1; IL-6, interleukin 6; MMP9, matrix metalloproteinase 9; CCL5, C-C chemokine ligand 5.

Results

NANOG Expression Positively Correlates with Cell Proliferation, Migration, and Cancer Stem-Like Characteristics in ESCC

We previously provided preliminary evidence that NANOG is highly expressed in ESCC, and that NANOG knockdown dramatically reduces cell proliferation.24 To clarify the role of NANOG in ESCC, we constructed an ectopic NANOG vector (pcDNA3.1-NANOG) and transfected different doses of ectopic NANOG in the Eca-109 cell line (ON cells). The specificity of pcDNA3.1-NANOG was verified using the rise in mRNA and protein levels of NANOG in different ESCC cell lines (Figure 1A and B). We found that the cell proliferation of Eca-109 cells positively correlated with the dose of ectopically expressed NANOG (Figure 1B). The total number of cells increased upon the overexpression of NANOG.

NANOG is an important transcription factor that maintains the pluripotency of ESCs. There is accumulating evidence that NANOG plays a critical role in tumorigenesis.25 In this study, we determined the roles of NANOG in ESCC migration and cancer stem-like properties. The sphere-forming assay was used to determine the relationship between the expression of NANOG and the sphere-forming ability in ESCC. In this experiment, we observed that the sphere-forming ability of ESCC was closely related to the expression of NANOG. Spheroid body formation increased significantly in the NANOG overexpression group (ON) and decreased in the knockdown group (shN1) (Figure 1C and F left). Moreover, as shown in Figure 1D and F (right), knocking down NANOG significantly reduced the ability of Eca-109 cells to migrate through Transwell pores, whereas overexpression of NANOG increased migration. These results indicate that NANOG expression is consistently required for ESCC cell proliferation, migration, and cancer stem-like properties (*P < .05; **P < .01; ***P < .001; ****P < .0001).

NANOG Promotes Cancer Cell Characteristics in ESCC by Activating IL-6/STAT3 Signaling

Cell proliferation, self-renewal, and EMT are regulated by various signaling pathways such as the TGF-β, Notch, STAT3, Wnt/β-catenin, and Hedgehog pathways.26 We examined whether NANOG is related to key factors in these signaling pathways. We found that the expression of IL-6 changed in a quantitative manner with the expression of NANOG (Figure 2A and B). The levels of IL-6 mRNA were determined using qPCR experiments (Figure 2C), and the level of IL-6 secreted into the growth medium was detected using an ELISA kit (Figure 2E). (*P < .05; **P < .01; ***P < .001). For most tumors, inflammation is a risk factor associated with tumor development and metastasis.26 The inflammatory cytokine IL-6 has been demonstrated to promote metastasis in a variety of tumor models.27 In addition, IL-6 increases the expression of anti-apoptotic proteins in ovarian cancer cells by activating the STAT3 signaling pathway.28

More importantly, studies have shown that Oct4 can regulate STAT3 expression in embryonic stem cells.29 Therefore, we hypothesized that NANOG, which is also an important stem cell regulatory transcription factor, plays a role in the cell proliferation, invasion, and CSC properties of ESCC by regulating IL-6/STAT3. Hence, we detected the expression of total STAT3 and p-STAT3 (Y705) in Eca-109 ON cells, Eca-109 CON (control) cells (Figure 2A and D), and Eca-109 shN1 cells (Figure 2B). Consistently, NANOG promoted cell proliferation and CSC properties through IL-6/STAT3 in TE-1 and KYSE-150 cells (Supplemental Figure S1A to D). The results showed that the expression of p-STAT3 and p-JAK2 protein positively correlated with NANOG expression, including the loss and overexpression conditions. However, NANOG expression had little effect on the total STAT3 level. These findings suggested that NANOG promoted cell proliferation, invasion, CSC properties, and resistance ability through IL-6/STAT3 activation in ESCC.
Target Genes of STAT3 are Differentially Expressed Upon NANOG Expression

Multiple genes associated with tumor growth and metastasis that are target genes of STAT3 play an important role in the occurrence and metastasis of tumors.\textsuperscript{30} We evaluated the related genes in Eca-109 cells under overexpression or knockdown of NANOG. Among these target genes, we chose a group of representative genes whose functions are known: Myelocytomatosis viral oncogene homolog (MYC), Bcl-xL, McI1 (apoptosis-related), Snail (migration and invasion-related), MMP9, CCL5 (resistance), CCND1 (cell cycle-related), and VEGFA (angiogenesis-related). The results showed that the mRNA expression of CCL5, CCND1, and VEGFA significantly increased in a dose-dependent manner when NANOG was overexpressed in Eca-109 cells (Figure 3A to C). It is notable that CCL5, CCND1, and VEGFA in most tumors are associated with the proliferation and invasion-ability genes and are significantly overexpressed.\textsuperscript{31-34} Consistently, Bcl-xL, McI1, MYC, MMP9, and Snail were differentially expressed upon NANOG expression (Figure 3D to H). Snail, CCL5, CCND1, MYC, and VEGFA levels increased upon NANOG overexpression, whereas MMP9 and McI1 levels changed inconsistently in TE-1 and KYSE-150 cells (Supplemental Figure S2A to G) (*P < .05; **P < .01; ***P < .001; ****P < .0001). These results revealed that diverse downstream target genes were dynamically regulated by NANOG expression via the IL-6/STAT3 pathway in ESCC.

Inhibition of IL-6/STAT3 Signaling Blocks Cell Proliferation, Invasion, and Cancer Stem-Like Properties in Eca-109 ON Cells

To further confirm whether NANOG promoted cell proliferation, invasion, and cancer stem-like properties via the IL-6/
STAT3 pathway, we blocked this pathway using antibodies of the IL-6 receptor or a STAT3 inhibitor (S31 to 201) in Eca-109 ON cells. We found that blocking of antibodies and inhibition of STAT3 phosphorylation both suppressed cell proliferation, cancer stem-like properties, and invasion effects in Eca-109 ON cells (Figure 4A to D) (**P < .01; ***P < .001).

These findings clearly indicated that the effects of NANOG were specifically due to IL-6/STAT3 signaling in ESCC.

Discussion

Previously, we have demonstrated that the pluripotent stem cell regulation factor NANOG is highly expressed in ESCC and participates in the development of esophageal cancer. In this work, our aim was to determine how NANOG implements its regulating role in ESCC. We constructed a vector for the ectopic expression of NANOG. We found that overexpression

Figure 2. NANOG promotes cancer cell characteristics in ESCC by activating IL-6/STAT3 signaling. (A and B) Expression of IL-6/STAT3, etc was detected by western blotting (A, overexpression and B, knockdown). (C) qPCR tests were used to determine the mRNA expression of NANOG and IL-6 for ectopic expression of different doses of NANOG. (D) Comparison of p-JAK2, total STAT3, p-STAT3, and IL-6 expression by immunoblotting for ectopic expression of different NANOG doses in Eca-109 cells. (E) The level of IL-6 secreted into the growth medium upon ectopic NANOG expression (lower panel) was detected using an ELISA kit, and compared with that of standard samples (upper panel) (*P < .05; **P < .01; ***P < .001).

Abbreviations: ESCC, esophageal squamous cell carcinoma; STAT3, signal transducer and activator of transcription 3; IL-6, interleukin 6; qPCR, quantitative real-time PCR; ELISA, enzyme-linked immunosorbent assay; p-JAK2, phospho-Janus kinase 2.
of NANOG promoted cell proliferation, invasion, and sphere formation in Eca-109 cells. It is notable that cell proliferation and invasion ability play an important role during tumor recurrence and metastasis. Consistently, the molecular experiments uncovered a novel mechanism: pluripotent transcription factor NANOG can act directly on tumor cell characteristics by activating IL-6/STAT3 signaling in ESCC. The data presented herein, combined with our previous results, reveal the function and mechanism of action of NANOG in ESCC. Inflammation is a risk factor in most tumors and is closely related to tumor progression and metastasis. When the IL-6 ligand binds to its receptor (coupled with gp130), signaling is activated. This binding leads to the activation of STAT3 by inducing the auto-phosphorylation and activation of Janus kinases (JAKs). In fact, changes in the downstream genes of STAT3 are the major causes of IL-6-related effects. The activation of STAT3 is important for normal cells, but the signal is strictly controlled. However, the abnormal expression of downstream genes (such as CCL5, CCND1, Snail, Twist, and VEGF) promotes cell proliferation and prevents apoptosis, whereas the activity of STAT3 signaling is out of control in various tumor cells. The expression of anti-apoptosis-related proteins

Figure 3. The downstream genes of STAT3 were differentially expressed upon ectopic NANOG expression. (A–H) qPCR tests were performed to evaluate the mRNA expression of multiple target genes of STAT3 from Eca-109 ON cell samples. (A) VEGFA; (B) CCL5; (C) CCND1; (D) Mcl1; (E) Snail; (F) JAK1; (G) MYC; (H) Bcl-xL. (*P < .05; **P < .01; ***P < .001; ****P < .0001).

Abbreviations: STAT3, signal transducer and activator of transcription 3; qPCR, quantitative real-time PCR; VEGFA, vascular endothelial growth factor A; CCND1, cyclin D1; CCL5, C-C chemokine ligand 5; JAK1, phospho-Janus kinase 1; MYC, myelocytomatosis viral oncogene homolog; Bcl-xL, B-cell lymphoma-extra-large.
has been proven to increase via the IL-6/STAT3 pathway in ovarian cancer cells. This study has demonstrated that the expression of IL-6 and p-STAT3 changes in a dose-dependent manner along with the expression of NANOG. Consistently, the downstream genes of STAT3, including CCL5, CCND1, and VEGF, were also differentially expressed upon NANOG expression. Strikingly, we found that blocking of the IL-6 receptor and inhibition of STAT3 phosphorylation both suppressed cell proliferation, cancer stem-like properties, and invasion effects in Eca-109 ON cells. In other words, NANOG promoted cell proliferation, invasion, and CSC properties via IL-6/STAT3 signaling in ESCC.

Upon further research, numerous studies have shown that noncoding RNAs such as long noncoding RNAs, microRNAs, and circular RNAs play an important role in gastrointestinal cancers, including esophageal cancer. Therefore, we hypothesize that NANOG, an important pluripotency-related transcription factor, affects the occurrence

![Figure 4](image-url)

**Figure 4.** Inhibition of IL-6/STAT3 blocked cell proliferation, invasion, and cancer stem-like properties in Eca-109 ON cells. (A) Microscopy of cell cloning of Eca-109 CON and ON cells with or without anti-IL-6 (N + S31 to 201) (upper panel: bright-field; lower panel: staining with Crystal Violet solution.) (B) Spheroid bodies derived from Eca-109 CON and ON cells with or without anti-IL-6 (N + S31 to 201). (C) Invasive ability of Eca-109 CON and ON cells with or without anti-IL-6 (N + S31 to 201) that were stained and photographed (100×). (D) Columnar analysis diagram of Eca-109 CON and ON cells with or without anti-IL-6 (N + S31 to 201). Cell numbers (left); spheroid body formation rate (center); and number of cells passing through the Matrigel (right) (**$P < .01$; ***$P < .001$). Abbreviations: STAT3, signal transducer and activator of transcription 3; IL-6, interleukin 6.
and development of esophageal cancer through the targeted regulation of different noncoding RNAs or as a target of different noncoding RNAs. This will be our next important research direction.

Authors’ Note
DL and GF designed the experiments, and DL and GF wrote the paper. DL, XPZ, XXC, RX, DQX, ZC, and KL performed the experiments.

Declaration of Conflicting Interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics Approval
In this study, commercial immortalized cell lines were used and no human or animal experiments were involved.

Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

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Supplemental Material
Supplemental material for this article is available online.

References
1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase. No. 11. Accessed March 24, 2017. http://globocan.iarc.fr/Default.aspx
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-132.
3. Hesari AR, Azizian M, Sheikhi A, et al. Chemopreventive and therapeutic potential of curcumin in esophageal cancer: current and future status. Int J Cancer. 2019;144(6):1215-1226.
4. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003;100(7):3983-3988.
5. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003;63(18):5821-5828.
6. Patrawala L, Calhoun T, Schneider-Brousard R, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene. 2006;25(12):1696-1708.
7. O’Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007;445(7302):106-110.
8. Boiko AD, Razorenova OV, van de Rijn M, et al. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature. 2010;466(7302):133-137.
9. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet. 2008;40(5):499-507.
10. Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY. Module map of stem cell genes guides creation of epithelial cancer stem cells. Cell Stem Cell. 2008;2(4):333-344.
11. Liu Y, Clem B, Zuba-Surma EK, et al. Mouse fibroblasts lacking RB1 function form spheres and undergo reprogramming to a cancer stem cell phenotype. Cell Stem Cell. 2009;4(4):336-347.
12. Pecce S, Tosoni D, Confalonieri S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell. 2010;140(1):62-73.
13. Chambers I, Tomlinson SR. The transcriptional foundation of pluripotency. Development. 2009;136(14):2311-2322.
14. Silva J, Nichols J, Theunissen TW, et al. Nanog is the gateway to the pluripotent ground state. Cell. 2009;138(4):722-737.
15. Ramadoss S, Sen S, Ramachandran I, Roy S, Chaudhuri G, Farias-Eisner R. Lysine-specific demethylase KDM3A regulates ovarian cancer stemness and chemoresistance. Oncogene. 2017;36(11):1537-1545.
16. Ling K, Jiang L, Liang S, et al. Nanog interaction with the androgen receptor signaling axis induce ovarian cancer stem cell regulation: studies based on the CRISPR/Cas9 system. J Ovarian Res. 2018;11(1):36.
17. Qin S, Li Y, Cao X, Du J, Huang X. Nanog regulates epithelial-mesenchymal transition and chemoresistance in ovarian cancer (BSR20160247). Biosci Rep. 2017;37:1-6.
18. Saga K, Park J, Nimura K, et al. Nanog helps cancer cells escape NK cell attack by downregulating ICAM1 during tumorigenesis. J Exp Clin Cancer Res. 2019;38(1):1-13.
19. Huang C, Yoon C, Zhou XH, et al. Erk1/2-Nanog signaling pathway enhances CD44+ cancer stem-like cell phenotypes and epithelial-to-mesenchymal transition in head and neck squamous cell carcinomas. Cell Death Dis. 2020;11(1):1-14.
20. Woo SR, Lee H-J, Oh SJ, et al. Stabilization of HDAC1 via TIC1-pAKT-CHFR axis is a key element for NANOG-mediated multi-resistance and stem-like phenotype in immune-edited tumor cells. Biochem Biophys Res Commun. 2018;503(1):1812-1818.
21. Kenda Šuster N, Frkovic Grazio S, Virant-Klun I, Verdenik I, Smrkolj Š. Cancer stem cell-related marker NANOG expression in ovarian serous tumors: a clinicopathological study of 159 cases. Int J Gynecol Cancer. 2017;27(9).
22. Iyer M, Mohana S, Jayaramayya Kaavya, Narayanasamy Arul, Vellingiri Balachandar, Kumaran S Santhy, New insight into NANOG: a novel therapeutic target for ovarian cancer (OC). Eur J Pharmacol. 2019;852(5):51-57.
23. Lu G Y, Yuey Li, Yanf Ma, et al. Long noncoding RNA LINC00511 contributes to breast cancer tumourigenesis and stemness by inducing the miR-185-3p/E2F1/Nanog axis. *J Exp Clin Cancer Res*. 2018;37(1):289.

24. Deng L, Xiang X, Yang F, et al. Functional evidence that the self-renewal gene NANOG regulates esophageal squamous cancer development. *Biochem Biophys Res Commun*. 2017;490(2):161-168.

25. Luis E, Santaliz-Ruiz IV, Xie X, Old M, Teknos TN, Pan Q. Emerging role of Nanog in tumorigenesis and cancer stem cells. *Int J Cancer*. 2014;135(12):2741-2748.

26. Bailey JM, Singh PK, Hollingsworth MA. Cancer metastasis facilitated by developmental pathways: sonic hedgehog, notch, and bone morphogenetic proteins. *J Cell Biochem*. 2007;102(4):829-839.

27. Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol*. 2014;26(1):54-74.

28. Sheng WJ, Jiang H, Wu DL, Zheng JH. Early responses of the STAT3 pathway to platinum drugs are associated with cisplatin resistance in epithelial ovarian cancer. *Braz J Med Biol Res*. 2013;46(8):650-658.

29. Guo Y, Mantel C, Hromas RA, Broxmeyer HE. Oct-4 is critical for survival/antiapoptosis of murine embryonic stem cells subjected to stress: effects associated with Stat3/survivin. *Stem Cells*. 2008;26(1):30-34.

30. Huang W, Liu Y, Wang J, et al. Small-molecule compounds targeting the STAT3 DNA-binding domain suppress survival of cisplatin-resistant human ovarian cancer cells by inducing apoptosis. *Eur J Med Chem*. 2018;157(5):887-897. 10.1016/j.ejmech.2018.08.037.

31. Zhang X, Yin P, Di D, et al. IL-6 regulates MMP-10 expression via JAK2/STAT3 signaling pathway in a human lung adenocarcinoma cell line. *Anticancer Res*. 2009;29(11):4497-4501.

32. Yuan G, Qian L, Shi M, et al. HER2-dependent MMP-7 expression is mediated by activated STAT3. *Cell Signal*. 2008;20(7):1284-1291.

33. Cheng GZ, Zhang WZ, Sun M, et al. Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. *J Biol Chem*. 2008;283(21):14665-14673.

34. Luwor RB, Styli SS, Kaye AH. The role of STAT3 in glioblastoma multiforme. *J Clin Neurosci*. 2013;20(7):907-911.

35. Razidlo GL, Burton KM, McNiven MA. Interleukin-6 promotes pancreatic cancer cell migration by rapidly activating the small GTPase CDC42. *J Biol Chem*. 2018;293(28):11143–11153.

36. Siddiquee KA, Gunning PT, Glenn M, et al. An oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing and induces antitumor cell effects. *ACS Chem Biol*. 2007;2(12):787-798.

37. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140(6):883-899.

38. Yan H, Guo BY, Zhang S. Cancer-associated fibroblasts attenuate Cisplatin-induced apoptosis in ovarian cancer cells by promoting STAT3 signaling. *Biochem Biophys Res Commun*. 2016;470(4):947-954.

39. Leila J, Roghayeh T, Sara T, et al. Circulating microRNAs as diagnostic and therapeutic biomarkers in gastric and esophageal cancers. *J Cell Physiol*. 2018;233(11):8538-8550.

40. Mhp A, Mv B, Mtc D, et al. Autophagy-related microRNAs: possible regulatory roles and therapeutic potential in and gastrointestinal cancers. *Pharmacol Res*. 2020;161:1–16.

41. Shafabakhsh R, Arianfar F, Vosough M, et al. Autophagy and gastrointestinal cancers: the behind the scenes role of long non-coding RNAs in initiation, progression, and treatment resistance. *Cancer Gene Ther*. 2021.

42. Naeli P, Pourhanifeh MH, Karimzadeh MR, et al. Circular RNAs and gastrointestinal cancers: epigenetic regulators with a prognostic and therapeutic role. *Crit Rev Oncol Hematol*. 2019;145:102854.