Research Article

MicroRNA-143-3p/TBX3 Axis Represses Malignant Cell Behaviors in Bladder Cancer

Lifu Huang,1,2 Xianjun Zhang,1,2 Feiping Li,1,2 and Xiaohong Wang2,3

1Department of Urology, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Linhai, Zhejiang Province, China 318050
2Enze Hospital, Taizhou Enze Medical Center (Group), Taizhou, Zhejiang Province, China 318050
3Obstetrical Department, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Linhai, Zhejiang Province, China 318050

Correspondence should be addressed to Xiaohong Wang; xiaoaohon@163.com

Received 27 October 2021; Revised 16 December 2021; Accepted 20 December 2021; Published 27 January 2022

Objective. To offer new insight for bladder cancer therapy through researching the microRNA-143-3p/TBX3 axis. Methods. Differentially expressed microRNAs in bladder cancer were provided by databases to find microRNA that may regulate TBX3. qRT-PCR was utilized to test levels of TBX3 mRNA and microRNA-143-3p. Their binding was verified with a dual-luciferase method. Malignant cell behaviors were examined by cell functional experiments. Levels of TBX3 protein and proteins pertinent to epithelial-mesenchymal transition (EMT) were tested by western blot. Results. TBX3 was highly expressed in bladder cancer cells. MicroRNA-143-3p presented the most conspicuously negative correlation with TBX3, and they had binding sites. Cell functional experiments proved that TBX3 facilitated bladder cancer cell functions and EMT. MicroRNA-143-3p was demonstrated to downregulate TBX3 expression. Rescue assay further illuminated that microRNA-143-3p repressed bladder cancer cell functions and EMT through downregulating TBX3 expression. Conclusion. These data all indicated that TBX3 was modulated by microRNA-143-3p and acted as a cancer promoter gene in bladder cancer progression via affecting tumor proliferation, migration, invasion, and EMT. Therefore, a microRNA-143-3p/TBX3 network might be an underlying target for bladder cancer.

1. Introduction

Bladder cancer is a prevalent malignancy in the urinary system [1]. Nonmuscle infiltrating bladder cancer comprises most of all bladder cancer cases (70%-80%) and shows a favorable prognosis [2]. Nonetheless, muscle infiltrating bladder cancer shows a terrible therapeutic effect, and the recurrence rate is high [3]. Generally, bladder cancer patients’ median survival is rather short (10-55 months). A patient’s poor prognosis often results from distant metastasis and high recurrence [4, 5], while epithelial-mesenchymal transition (EMT) facilitates invasiveness and activity of bladder cancer cells [6, 7]. Hence, it is critical to define the functional mechanism in the early stage of metastasis, such as migration, invasion, and EMT.

T-box protein family consists of a group of evolved conservative transcription factors which modulate levels of genes [8]. They work as transcriptional inhibitors or activators to modulate EMT, tissue integrity, and cell differentiation [9–11]. Overexpressing T-box factors such as TBX3 and TBX2 might trigger cancers [12–16], and these two transcription factors are activated in melanoma [17, 18] and bladder cancer [19, 20] and are proved to be necessary for tumorigenesis and migration [20, 21]. Furthermore, previous studies suggested that TBX3 expression promotes tumor EMT and TBX3 upregulation directly inhibits adhesion molecule E-cadherin expression, thereby increasing the aggressiveness of melanoma [22]. E-cadherin is an important medium of cell-cell interaction, which is verified to be significant in EMT, and loss of E-cadherin is the most
crucial marker for EMT occurrence [23]. TBX3 is proved to promote proliferation and invasion and repress apoptosis of rat bladder cancer cells [24]. Conspicuously expressed TBX3 functions as an underlying indicator for bladder cancer diagnosis and/or prognosis [19]. An intriguing investigation also supports this result by identifying TBX3/TBX2 as a favorable marker for primary pTaG1/2 bladder cancer [25]. All these data lay stress on the importance of TBX3 as a potential biomarker. Nonetheless, how TBX3 functioned in the malignant progression of bladder cancer has been hardly known.

TBX3 was predicted as a target of microRNA-143-3p through bioinformatics methods. Therefore, this investigation is concerned with microRNA-143-3p/TBX3 in bladder cancer and the effect of their abnormal expression on tumor biological functions. Our results may lay a foundation for finding potential molecular therapeutic targets of bladder cancer.

2. Materials and Methods

2.1. Cell Culture. Human bladder cancer cell lines T24 (BNCC311582), BIU-87 (BNCC100982), and immortalized human bladder epithelial cell line SV-HUC-1 (BNCC100273) were bought from Bena Culture Collection (BNCC) (China). Human bladder cancer cell line UMC3 (ATCC® CRL-1749™) was from American Type Culture Collection (ATCC) (USA). T24 and BIU-87 cell lines were treated in RPMI-1640 medium plus 10% fetal bovine serum (FBS). UMC3 cell line was placed in DMEM with 10% FBS. SV-HUC-1 was cultured in corresponding mediums with 5% CO2 at 37°C. All cell lines were cultured in a temperature incubator with 5% CO2 at 37°C.

2.2. Construction of Vectors and Transfection of Cells. MicroRNA-143-3p mimic, mimic NC, si-TBX3, si-NC, oe-TBX3, and oe-NC were designed by Guangzhou RiboBio. Lipofectamine 2000 (Thermo Fisher Scientific) was applied to transiently transfect synthesized sequences or plasmids into bladder cancer cells T24 or BIU-87. Cells were incubated at least 24 h and needed to be washed with phosphate-buffered saline (PBS) (pH 7.4).

2.3. qRT-PCR. Total RNA was isolated from cells using TRIzol kit (Invitrogen, Carlsbad, CA, USA). RNA concentration was determined by NanoDrop 2000 system (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Following the kit instruction, miScript IIRT kit (Qiagen, USA) was used to reversely transcribe microRNAs into cDNA, and mRNAs were reversely transcribed into cDNA by using PrimeScript RT Master Mix (Takara Bio Inc., Dalian, P.R. China). miScript SYBR Green PCR Kit (Qiagen, Germany) was used to detect microRNA expression and SYBR® Premix Ex Taq™ II (Takara Bio Inc., Shiga, Japan) was applied to detect mRNA expression. qRT-PCR was undertaken on Applied Biosystems®7500 Real-Time PCR Systems (Thermo Fisher Scientific, Inc., Waltham, MA). Table 1 provides the used primers that were all purchased from Sangon Biotech (Shanghai, China). U6 and β-actin were taken as the internal references of microRNA-143-3p and TBX3, respectively. The difference of relative expression between groups was compared by 2^-ΔΔCt value.

2.5. Cell Counting Kit- (CCK-8) 8 and Colony Formation Assays. In CCK-8, 2×10^4 transfected cancer cells were placed into 96-well plates with each well (100 μl medium). At 0, 24, 48, 72, and 96 h, 10 μl CCK-8 (Dojindo, Tokyo, Japan) was added for another 1 h of incubation. Absorbance was measured with a microplate reader at 450 nm. In colony formation assay, transfected bladder cancer cells were inoculated into 6-well plates at each well (1×10^4) cells. Next, cells of each well were cultured in 2 ml medium for a week. Afterward, cells were washed with PBS twice, treated with 4% paraformaldehyde and 0.1% crystal violet for 20 min. Aggregates ≥ 50 cells (diameter: 0.3 mm-1 mm) were defined as colonies.

2.6. Detection of Migration and Invasion. Cell migration and invasion were examined by using Transwell cell migration
chamber and BioCoat Matrigel-coated Transwell cell invasion chamber (Corning, NY, USA). After 24 h of transfection, T24 or BIU-87 cells were suspended in 200 μl serum-free medium. Cell suspension was added into the upper chamber, and the lower chamber was covered with 500 μl RPMI-1640 containing 15% FBS. Thereafter, cells were incubated at 37°C for 24 h. Next, cells in the upper chamber were removed. Cells in the lower chamber were treated with 4% paraformaldehyde for fixation and 0.5% crystal violet for staining. Under 5 randomly selected fields of the microscope (100x), migrating and invading cells were, respectively, counted.

2.7. Dual-Luciferase Reporter Detection. To identify the binding between microRNA-143-3p and TBX3 3′-UTR, mutant (MUT) (by site mutation), and wild-type (WT) TBX3 3′-UTR were constructed. Next, sequences were inserted into psiCHECK luciferase reporter plasmids (Sangon Co., LTD, Shanghai, China). Next, bladder cancer cells (T24) were inoculated in 48-well plates and cultured at 37°C for 24 h. T24 cells were transfected with microRNA-143-3p mimic or mimic NC and WT/MUT-TBX3 plasmids. Ultimately, luciferase activity was measured through a luciferase assay kit (Promega, Fitchburg, WI, USA).

2.8. Statistical Analysis. Data were exhibited as the mean ± standard deviation (SD) and processed by using GraphPad Prism 6.0 software (GraphPad Inc., San Diego, CA, USA). The difference between two groups was analyzed by a t-test and among more than 2 groups was analyzed by variance analysis. All experiments were repeated 3 times independently. p < 0.05 was defined as statistically significant.

3. Results

3.1. TBX3 Is Conspicuously Highly Expressed in Bladder Cancer. Combining reference analysis, TBX3 overexpression is relevant to many cancers, such as liver cancer, pancreatic cancer, ovarian cancer, and head and neck squamous cell carcinoma [26]. However, little is reported about the mechanism by which TBX3 modulates bladder cancer, and therefore, we chose TBX3 for research. Basic TBX3 level in T24, BIU-87, UMUC3, and SV-HUC-1 was tested first. It was unveiled that TBX3 expression was higher in cancer cells than in normal cells at mRNA and protein levels (Figures 1(a) and 1(b)). Hence, we considered that TBX3 was remarkably upregulated in bladder cancer. T24 and BIU-87 cells with a remarkable difference in TBX3 expression were used for the following experiments.

3.2. TBX3 Facilitates Bladder Cancer Cell Proliferation, Migration, Invasion, and EMT. Since TBX3 was conspicuously highly expressed in bladder cancer cells, this part is aimed at verifying its effect on the biological function of bladder cancer cells. Cells with TBX3 low expression and TBX3 high expression were constructed by transient transfection of si-TBX3 (si-NC as control) into T24 cells and oe-TBX3 (oe-NC as control) into BIU-87 cells, respectively. qRT-PCR was applied to detect transfection efficiency (Figure 2(a)). CCK-8 and colony formation assays indicated that silenced TBX3 remarkably inhibited T24 cell proliferation, while overexpressed TBX3 facilitated BIU-87 cell proliferation (Figures 2(b) and 2(c)).

Subsequent analyses were conducted to determine whether TBX3 impacted cancer invasion and migration. Transwell assay suggested that declined TBX3 expression suppressed T24 cell migration and invasion, while overexpressed TBX3 increased BIU-87 cell migration and invasion (Figure 2(d)). Furthermore, EMT-related proteins in different transfection groups were measured by western blot. An increase in E-cadherin level while a reduction in levels of N-cadherin and Vimentin was observed in si-TBX3-transfected T24 cells, and TBX3-overexpressed BIU-87 cells showed the opposite situation (Figure 2(e)).

3.3. MicroRNA-143-3p Downregulates TBX3 Expression. To further determine how TBX3 affects the malignant progression of bladder cancer, we predicted its upstream microRNAs. The predicted microRNAs by bioinformatics databases were intersected with differentially expressed microRNAs (DEmicroRNAs) from TCGA to acquire 7 candidate microRNAs (Figure 3(a)). Afterward, correlation analysis was conducted on the 7 microRNAs and TBX3, and it was displayed that microRNA-143-3p had the most
2.5
1.0
1.5
2.0
0.5

BIU-87
T24

Relative expression of TBX3
oe-NC oe-TBX3si-TBX3si-NC

⁎⁎⁎
⁎⁎

(a)

Absorbance (OD450 nm)

TIME (h)

0 24 48 72 96

2.0
2.0
1.0
1.0
1.5
1.5
0.5
0.5

si-TBX3
si-NC

oe-TBX3
oe-NC

(b)

C0lony number

T24

0 50 100 150

si-NC si-TBX3

⁎⁎⁎
⁎⁎

(c)

Absorbance (OD450 nm)

TIME (h)

0 24 48 72 96

Figure 2: Continued.
significantly negative correlation with TBX3 (Figure 3(b)). Meanwhile, it was predicted that they had binding sites (Figure 3(c)). A dual-luciferase method confirmed that microRNA-143-3p overexpression declined luciferase intensity of WT TBX3 3'UTR reporter plasmids, suggestive of their direct binding (Figure 3(d)). Thereafter, qRT-PCR discovered markedly low microRNA-143-3p levels in cancer cells (Figure 3(e)). Moreover, we constructed microRNA-143-3p overexpressed T24 cells and NC T24 cells. TBX3 expression in two groups was compared. Results exhibited that TBX3 mRNA and protein level in microRNA-143-3p mimic transfected T24 cells were significantly downregulated.
Overall, microRNA-143-3p was less expressed in bladder cancer and had negatively regulatory relationship with TBX3.

3.4. MicroRNA-143-3p Constrains EMT and Cell Functions via TBX3 Mediation. In the above context, we testified that microRNA-143-3p could downregulate TBX3 expression. Thereafter, to further scrutinize the microRNA-143-3p/TBX3 axis, we arranged rescue assays. MicroRNA-143-3p mimic was utilized to recover TBX3 expression (Figure 4(a)). As revealed by CCK-8 and colony formation methods, overexpression of TBX3 alone markedly stimulated cancer cell proliferation and colony formation, whereas overexpressing it and microRNA-143-3p simultaneously was able to counteract such promoting impact (Figures 4(b) and 4(c)).

Transwell also demonstrated that microRNA-143-3p led to a reduction of TBX3-triggered cancer cell migration and invasion (Figure 4(d)). Based on western blot, TBX3 overexpression alone declined E-cadherin expression while elevating Vimentin and N-cadherin expression, hinting that those epithelial cells acquired mesenchymal properties. Nevertheless, such properties were reversed by microRNA-143-3p
Relative expression of TBX3

| Condition               | Expression |
|-------------------------|------------|
| mimic NC+oe-NC          | 0.0        |
| mimic NC+oe-TBX3        | 0.5        |
| miR-143-3p mimic+oe-TBX3| 2.0        |

Absorbance (OD450 nm)

| TIME (h) | Absorbance |
|----------|------------|
| 0        | 0.0        |
| 24       | 0.5        |
| 48       | 1.0        |
| 72       | 1.5        |
| 96       | 2.0        |

(a)

(b)

Colony number

| Condition               | Colony number |
|-------------------------|---------------|
| mimic NC+oe-NC          | 200           |
| mimic NC+oe-TBX3        | 150           |
| miR-143-3p mimic+oe-TBX3| 100           |

(c)

(d)

Cell number

| Condition               | Cell number |
|-------------------------|-------------|
| mimic NC+oe-NC          | 300         |
| mimic NC+oe-TBX3        | 200         |
| miR-143-3p mimic+oe-TBX3| 100         |

Figure 4: Continued.
mimic (Figure 4(e)). Altogether, microRNA-143-3p restrained bladder cancer cell functions and EMT through downregulating TBX3.

4. Discussion

Tumor invasion and metastasis are complex processes involving many genes and steps [27]. EMT is one of the important behaviors of tumor metastasis [28, 29]. EMT is a biological process that greatly increases the invasion and metastasis of malignant tumors [30]. EMT mainly occurs in epithelial cell cancers, and therefore, it also regulates bladder cancer. In this context, we explored a molecular biomarker that may help us further understand the metastasis and invasion of bladder cancer in this study.

There is a study that proved that TBX3 is differentially upregulated in bladder cancer [20]. TBX3 expresses in any type of tissue during embryonic development and functions as a transcriptional inhibitor [31]. TBX3 is also involved in various carcinogenic processes, such as proliferation, migration, and invasion [32–34]. Moreover, TBX3 is closely relevant to EMT [35]. TBX3 can facilitate preinvasive breast cancer cell progression via stimulating EMT and upregulating SLUG [35]. TBX3 expression level is upregulated in many cancers, but most references focus on breast cancer instead of bladder cancer [36–38]. Hence, TBX3 was chosen for research. EMT-related protein expression level and cell biological behaviors were detected here by western blot and cell functional experiments, and the results verified the promotion of TBX3 on bladder cancer cell migration, invasion, and EMT. This indicated a good agreement between the above experiments and references that TBX3 acts as a promoter and is relevant to EMT in bladder cancer.

To further determine how TBX3 modulates bladder cancer progression, we predicted its upstream microRNA and performed correlation analysis. At last, microRNA-143-3p that had binding sites and was negatively correlated with TBX3 was obtained. MicroRNA-143-3p affects the development of various cancers as a tumor suppressor [39]. MicroRNA-143-3p expression is remarkably low and inhibits cell proliferation in colon cancer [40]. In breast cancer, microRNA-143-3p is less expressed which restrains the proliferation of breast cancer and stimulates apoptosis [41]. Similar phenomenon has also been observed in bladder cancer. SNHG1 is capable of endogenously sponging microRNA-143-3p in the cytoplasm of bladder cancer cells to affect cell proliferation and motor ability [42]. Likewise, we proved that microRNA-143-3p was notably less expressed and inhibited TBX3 expression in bladder cancer through experiments. In addition, rescue assay further presented that microRNA-143-3p overexpression downregulated TBX3 expression to mediate cancer cell migration, invasion, and EMT. EMT allows cancer cells to be equipped with mesenchymal properties to escape from primary tumors [43]. N-cadherin mediates signal transduction compound, loss of which is a clinical indicator of bad prognosis and metastasis [27]. It was postised that microRNA-143-3p/TBX3 axis hampered EMT to hinder bladder cancer migration and invasion. Such molecular mechanism was verified for the first time.

On the above, the microRNA-143-3p/TBX3 axis is closely related to bladder cancer migration, invasion, and EMT as evidenced by bioinformatics, molecular, and cellular
analyses. In the future, we plan to further confirm our findings by animals and clinical trials and expect to provide more solid and perfect theoretical supports for bladder cancer diagnosis and treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

Authors’ Contributions

All authors contributed to data analysis, drafting, and revising the article; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All authors consent to submit the manuscript for publication.

Acknowledgments

This study was supported by the funds from Taizhou Science and Technology Bureau Class A +1801ky76.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit et al., “Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012,” *International Journal of Cancer*, vol. 136, no. 5, pp. E359–E386, 2015.

[2] F. Sanguedolce, P. Bufo, G. Carrieri, and L. Cormio, “Predic- tive markers in bladder cancer: do we have molecular markers ready for clinical use?,” *Critical Reviews in Clinical Laboratory Sciences*, vol. 51, no. 5, pp. 291–304, 2014.

[3] M. Rouprêt, M. Babjuk, E. Compérat et al., “European guidelines on upper tract urothelial carcinomas: 2013 update,” *European Urology*, vol. 63, no. 6, pp. 1059–1071, 2013.

[4] J. Alfred Witjes, T. Lebret, E. M. Compérat et al., “Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer,” *European Urology*, vol. 71, no. 3, pp. 462–475, 2017.

[5] D. Fang, L. Zhang, X. Li et al., “Presence of concomitant non-muscle-invasive bladder cancer in Chinese patients with upper tract urothelial carcinoma: risk factors, characteristics, and predictive value,” *Annals of Surgical Oncology*, vol. 22, no. 8, pp. 2789–2798, 2015.

[6] D. J. McConkey, W. Choi, L. Marquis et al., “Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer,” *Cancer Metastasis Reviews*, vol. 28, no. 3–4, pp. 335–344, 2009.

[7] Q. Xie, T. Tang, J. Pang et al., “LSD1 promotes bladder cancer progression by upregulating LEF1 and enhancing EMT,” *Frontiers in Oncology*, vol. 10, p. 1234, 2020.

[8] J. Lu, X. P. Li, Q. Dong, H. F. Kung, and M. L. He, “TBX2 and TBX3: the special value for anticancer drug targets,” *Biochimica et Biophysica Acta*, vol. 1806, no. 2, pp. 268–274, 2010.

[9] Y. Yan, M. Su, Y. Song et al., “Tbx1 modulates endodermal and mesodermal differentiation from mouse induced pluripotent stem cells,” *Stem Cells and Development*, vol. 23, no. 13, pp. 1491–1500, 2014.

[10] V. E. Papaioannou, “The T-box gene family: emerging roles in development, stem cells and cancer,” *Development*, vol. 141, no. 20, pp. 3819–3833, 2014.

[11] R. I. Fernando, M. Litzinger, P. Trono, D. H. Hamilton, J. Schlom, and C. Palena, “The T-box transcription factor bra-chyury promotes epithelial-mesenchymal transition in human tumor cells,” *The Journal of Clinical Investigation*, vol. 120, no. 2, pp. 533–544, 2010.

[12] J. Peres and S. Prince, “The T-box transcription factor, TBX3, is sufficient to promote melanoma formation and invasion,” *Molecular Cancer*, vol. 12, no. 1, p. 117, 2013.

[13] B. Bilićan and C. R. Goding, “Cell cycle regulation of the T-box transcription factor tbx3,” *Experimental Cell Research*, vol. 312, no. 12, pp. 2358–2366, 2006.

[14] D. Burgucu, K. Guney, D. Sahinturk et al., “Tbx3 represses PTEN and is over-expressed in head and neck squamous cell carcinoma,” *BMC Cancer*, vol. 12, no. 1, p. 481, 2012.

[15] W. M. Hoogaars, P. Barnett, M. Rodriguez et al., “TBX3 and its splice variant TBX3 + exon 2a are functionally similar,” *Pigment Cell & Melanoma Research*, vol. 21, no. 3, pp. 379–387, 2008.

[16] J. O. Humtsoe, E. Koya, E. Pham et al., “Transcriptional profil- ing identifies upregulated genes following induction of epithelial-mesenchymal transition in squamous carcinoma cells,” *Experimental Cell Research*, vol. 318, no. 4, pp. 379–390, 2012.

[17] K. Hoek, D. L. Rimm, K. R. Williams et al., “Expression profil- ing reveals novel pathways in the transformation of melanocytes to melanomas,” *Cancer Research*, vol. 64, no. 15, pp. 5270–5282, 2004.

[18] J. Peres, S. Mowla, and S. Prince, “The T-box transcription fac- tor, TBX3, is a key substrate of AKT3 in melanomagenesis,” *Oncotarget*, vol. 6, no. 3, pp. 1821–1833, 2015.

[19] S. Amini, F. Fathi, J. Mobalegi, H. S. Soja et al., “Expression of Tbx2 is associated with cell proliferation and decreased apoptosis in bladder cancer,” *Mol Biol Rep*, vol. 39, no. 2, pp. 323–330, 2012.

[20] J. J. Jacobs, P. Keblusek, E. Robanus-Maandag et al., “Expression in bladder cancer cell lines,” *European Urology*, vol. 63, no. 6, pp. 1059–1071, 2013.

[21] J. P. Thiery, T. Ghadimi, and C. Palena, “Epithelial-mesenchymal transition from mouse induced pluripotent stem cells, and its clinical relevance,” *Cancer Metastasis Reviews*, vol. 34, no. 3–4, pp. 335–344, 2009.

[22] M. Rodriguez, E. Aladowicz, L. Lanfrancone, and C. R. God- ing, “TBX3 represses E-cadherin expression and enhances mel- anoma invasiveness,” *Cancer Research*, vol. 68, no. 19, pp. 7872–7881, 2008.

[23] J. P. Thiery, “Epithelial-mesenchymal transitions in tumour progression,” *Nature Reviews. Cancer*, vol. 2, no. 6, pp. 442–454, 2002.

[24] A. Ito, M. Asamoto, N. Hokiawi, S. Takahashi, and T. Shirai, “Tbx3 expression is related to apoptosis and cell prolif- eration in rat bladder both hyperplastic epithelial cells and...
carcinoma cells,” *Cancer Letters*, vol. 219, no. 1, pp. 105–112, 2005.

[25] W. Beukers, R. Kandimalla, R. G. Masius et al., “Stratification based on methylation of *TBX2* and *TBX3* into three molecular grades predicts progression in patients with pTa-bladder cancer,” *Modern Pathology*, vol. 28, no. 4, pp. 515–522, 2015.

[26] L. Dong, X. Lyu, O. D. Faleti, and M. L. He, “The special stemness functions of Tbx3 in stem cells and cancer development,” *Seminars in Cancer Biology*, vol. 57, pp. 105–110, 2019.

[27] M. Yilmaz and G. Christofori, “TBX3 is overexpressed in breast cancer and represses p14 ARF by interacting with histone deacetylases,” *Cancer Research*, vol. 68, no. 3, pp. 693–699, 2008.

[28] M. V. Iorio, M. Ferracin, C. G. Liu et al., “MicroRNA gene expression deregulation in human breast cancer,” *Cancer Research*, vol. 65, no. 16, pp. 7065–7070, 2005.

[29] R. Kalluri and R. A. Weinberg, “The oncogenic T-box factor TBX3 is important in S-phase and is regulated by c-Myc and cyclin A-CDK2,” *Cell Cycle*, vol. 14, no. 9, pp. 3173–3183, 2015.

[30] J. Li, M. S. Weinberg, L. Zerbini, and S. Prince, “The long non-coding RNA SNHG1 promotes bladder cancer progression by interacting with miR-143-3p and EZH2,” *Journal of Cellular and Molecular Medicine*, vol. 24, no. 20, pp. 11858–11873, 2020.

[31] C. Rallis, J. del Buono, and M. P. O. Logan, “The basics of epithelial-mesenchymal transition,” *The Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.

[32] S. Wansleben, J. Peres, S. Hare, C. R. Goding, and S. Prince, “T-box transcription factors in cancer biology,” *Biochimica et Biophysica Acta*, vol. 1846, no. 2, pp. 380–391, 2014.

[33] H. Zhu, U. Dougherty, V. Robinson et al., “EGFR signals downregulate tumor suppressors miR-143 and miR-145 in Western diet-promoted murine colon cancer: role of G1 regulators,” *Molecular Cancer Research*, vol. 9, no. 7, pp. 960–975, 2011.

[34] Y. L. Tuo, X. M. Li, and J. Luo, “Long noncoding RNA UCA1 modulates breast cancer cell growth and apoptosis through decreasing tumor suppressive miR-143,” *European Review for Medical and Pharmacological Sciences*, vol. 19, no. 18, pp. 3403–3411, 2015.

[35] W. Xiang, L. Lyu, T. Huang et al., “The special stem-mesenchymal transition regulator Slug in circulating tumor cells from pancreatic cancer patients: potential role in clinical practice,” *World Journal of Gastroenterology*, vol. 25, no. 1, pp. 138–150, 2019.

[36] C. C. Alves, F. Carneiro, H. Hoefler, and K. F. Becker, “Role of the epithelial-mesenchymal transition marker Slug in normal development and tumor progression,” *Molecular Cancer Research*, vol. 119, no. 6, pp. 1428, 2009.

[37] R. Kalluri and R. A. Weinberg, “The basics of epithelial-mesenchymal transition,” *Cancer Metastasis Reviews*, vol. 28, no. 1–2, pp. 15–33, 2009.

[38] S. Souchelnytskyi, “The basics of epithelial-mesenchymal transition markers in circulating tumor cells from pancreatic cancer patients: potential role in clinical practice,” *World Journal of Gastroenterology*, vol. 25, no. 1, pp. 138–150, 2019.

[39] C. Rallis, J. del Buono, and M. P. O. Logan, “The T-box factor TBX3 is important in S-phase and is regulated by c-Myc and cyclin A-CDK2,” *Cell Cycle*, vol. 14, no. 9, pp. 3173–3183, 2015.

[40] J. Li, M. S. Weinberg, L. Zerbini, and S. Prince, “The oncogenic T-box factor TBX3 is important in S-phase and is regulated by c-Myc and cyclin A-CDK2,” *Cell Cycle*, vol. 14, no. 9, pp. 3173–3183, 2015.

[41] C. Rallis, J. del Buono, and M. P. O. Logan, “The oncogenic T-box factor TBX3 is important in S-phase and is regulated by c-Myc and cyclin A-CDK2,” *Cell Cycle*, vol. 14, no. 9, pp. 3173–3183, 2015.