Iran J Public Health, Vol. 52, No.2, Feb 2023, pp.306-314

Original Article

Forkhead box A2 Promotes Colorectal Cancer Progression and Targets BCL2-Associated X (BAX) Protein

Xiangyue Zeng, Xinhui Yang, Shayim Parhatsayim, Abulajiang Abudoukelimu, Yin Shu, *Zeliang Zhao

Department of Gastrointestinal Surgery (Inpatient Area 2), Cancer Hospital of Xinjiang Medical University, Urumqi, Xinjiang, 830011, China

***Corresponding Author:** Email: zxy20210912@163.com

(Received 22 Jul 2022; accepted 12 Sep 2022)

Abstract

Background: Colorectal cancer is the third most common malignant tumor in the world and substantial death cases are reported each year. We aimed to explore the molecular mechanism underlying colorectal cancer tumorigenesis and progression.

Methods: The expression levels of *Forkhead box A2* (*FOXA2*) in colorectal cancer tissues were first analyzed using Gene Expression Profiling Interactive Analysis (GEPIA). More multiple in vitro experiments established the role of *FOXA2* in colorectal cancer progression. The potential downstream target of *FOXA2* was identified by Western blot analysis.

Results: FOXA2 expression level was significantly up-modulated in colorectal cancer specimens and cells (P<0.05). Silencing *FOXA2* remarkably inhibited colorectal cancer cells growth, invasion and migration. BCL2-associated X (BAX) protein was identified as a potential downstream protein of *FOXA2*.

Conclusion: Our findings demonstrated the essential role of *FOXA2* in colorectal cancer progression and identified BAX protein as its potential target.

Keywords: Colorectal cancer; FOXA2 protein; bcl-2-associated X protein; Invasion; Migration

Introduction

Colorectal cancer (CRC) accounts for approximately 10% of all annually new cancer cases and deaths in the world, with 1.2 million new cases and almost 0.9 million deaths reported each year (1-3). Despite continuing progress in CRC treatments in the past few decades, the prognosis for patients with CRC is still grim, especially for those at an advanced stage. Although genetic, lifestyle, and environmental factors were reported to associate with CRC initiation and progression, the exact reasons were not completely clear (1). Therefore, investigating the molecular mechanism underlying CRC tumorigenesis and development is a promising area of further study for possible therapies.

The human *Forkhead box (FOX)* gene superfamily has crucial roles in a series of biological processes (4). The transcription factor Forkhead box A2 (*FOXA2*), a member of this family, has been implicated in many cellular processes such as em-

Copyright © 2023 Zeng et al. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license.
(https://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited

bryonic development, metabolism and homeostasis (4-7). *FOXA2* is also involved in the regulation of cancer development. Tumor-suppressing effects of *FOXA2* have been discovered in lung cancer (8, 9), prostate cancer (10, 11), breast cancer (12) and liver cancer (13, 14). However, *FOXA2* was overexpressed in breast cancer cells. Besides, it maintains self-renewal property of cancer stem cells and positively correlates with tumor recurrence (15). Additionally, *FOXA2* participates in the regulation of transcription factors expression, leading to inhibition or promotion of cell proliferation, migration, differentiation and autophagy in multiple cancer types (16-18). Given that essential roles of *FOXA2* in above processes, investigating *FOXA2* function is of great significance.

BCL2-associated X protein (BAX), an indispensable member of B-cell lymphoma 2 (Bcl-2) family, widely described as a pro-apoptotic factor, plays a cardinal role in the late signaling phase of programmed cell death (19). A high level of BAX expression contributes to cell apoptosis via mitochondrial membrane damage and subsequently the release of cytochrome c upon stimulation of cytotoxic factors or cellular stress (20). Dysregulation of BAX protein levels is frequently observed in various cancers such as colon cancer (21), breast cancer (22), and ovarian cancer (23). Above phenomenon reveals the anti-tumor potential of BAX and the possibility of BAX-targeted strategy.

We aimed to examine the function of *FOXA2* in CRC. Then we sought to investigate whether the protein levels of the target genes of *FOXA2* established in the previous study (24) are affected after *FOXA2* downregulation.

Materials and Methods

Gene Expression Analysis Using GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/index.html), an interactive online tool for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov/) and Genotype-Tissue Expression (GTEx; https://www.gtexportal.org/home/datasets)(25), was used to analyze *FOXA2* expression levels in the colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ).

Cell culture

Human colorectal cancer cell lines (HT-29, SW620, Caco-2, HCT116, and SW480) were obtained from the American Type Culture Collection (Manassas, VA, USA), and the normal human colonic epithelial cell line NCM460 was purchased from In Cell (San Antonio, TX, USA). These entire cell lines were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 at 37 ℃.

Generation of stable cell lines

Lentiviral delivery of a shRNA sequence was used to knock down *FOXA2* expression (26). Caco-2 and HCT116 cells stably expressing shFOXA2 or shCtrl were constructed using a lentiviral packaging kit (Santa Cruz Biotechnology) and selected with 5 mg/ml puromycin 48h after transfection. All procedures were performed under the manufacturer's instructions. The shRNA sequences were listed as follows: FOXA2 shRNA#1: 5'- GAACGGCATGAACAC-GTACAT-3'; FOXA2 shRNA#2: 5'-CAAGGGAGAAGAAATCCATA-3';

Western blot analysis

Western blot was used to assess protein expression levels as previously described (27). Whole cells were harvested and lysed using RIPA (Beyotime) buffer containing a protease inhibitor cocktail (Roche) and PMSF (Roche). Proteins were fractionated by 10%–15% SDS–polyacrylamide gel, subsequently electrophoretically transferred to 0.40 mm polyvinylidene difluoride membranes. The membranes were blocked with a mixture of 5% nonfat milk and PBS-Tween 20 for 1 hour at room temperature,

incubated with specific primary antibodies overnight at 4 ℃, followed by incubation with a horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology).

CCK-8 Assay

The cell viability was detected by using Cell Counting Kit-8 (CCK-8) according to the manufacturer's protocols. Briefly, cells (2×10^3 cells/well) were seeded in 96-well plates and cultured. Ten μl CCK-8 reagent was added per well and cell proliferation was detected at the indicated time points. The absorbance at 450 nm was measured for each sample. All experiments were conducted in triplicate and repeated at least three times.

Colony Formation Assay

The cells (5×10^2 cells/well) were seeded in a 60-mm tissue culture plate and grown for 14 days. The visible colonies were fixed with 4% paraformaldehyde followed by being stained with 1% crystal violet overnight. After washing with PBS thrice, the colonies were counted and pictured.

Invasion Assay

The 24-well BD Matrigel invasion chambers (BD Biosciences) were utilized to investigate CRC cells invasion under the manufacturer's instructions. Briefly, 2 CRC cell lines were resuspended in DMEM without serum and added to the upper chamber wells pre-coated with Matrigel. The lower chamber wells were filled with DMEM containing 20% FBS, as a chemoattractant. After 24 h incubation, invading cells were fixed with 100% methanol for 30 min, stained with 0.1% crystal violet for 20 min, and photographed in three independent 10 × magnification fields.

Wound Healing Assay

Cells (3×10^5 cells/well) were seeded into 6-well plates and cultured. The monolayer was scratched by using a sterile 20-μl pipette tip after the cells reached 90% confluence. The plates were washed twice using PBS to remove floating cells followed by serum-free medium added. The scratched areas were photographed at the indicated time points by a light microscope (Leica, Germany). The migration index was then calculated in terms of wound coverage.

Statistical Analysis

The statistical analyses in this study were performed by using GraphPad Prism 7. All experimental data was expressed in the format of mean ± standard deviation. The significance of differences between two paired groups was determined by Student's t test. One-way analysis of variance was used for multiple group comparison. $P < 0.05$ was considered statistically significant.

Results

FOXA2 expression is significantly upregulated in CRC samples and cell lines

The results of GEPIA showed that the level of *FOXA2* was substantially upregulated in READ and COAD compared with their corresponding normal tissues ($P< 0.05$, Fig. 1A). As shown in Fig. 1B, all tested CRC cell lines had dramatically higher FOXA2 protein levels than those in the NCM460, with the exception of HT29, which presented no significant difference.

FOXA2 promotes the growth of CRC cells

Firstly, the significantly reduced expression of *FOXA2* in Caco-2 and HCT116 cells confirmed the knockdown efficiency of *FOXA2* shRNAs (Fig. 2A). CCK-8 assay showed that *FOXA2*-depleted Caco-2 and HCT116 cells exhibited remarkably lower growth rates in comparison with control cells (Fig. 2B). Consistent with this, colony formation assay also revealed that the number of colonies was significantly decreased after 12 days of culture when *FOXA2* expression was inhibited in comparison to the controls ($P< 0.05$, Fig. 2C, 2D).

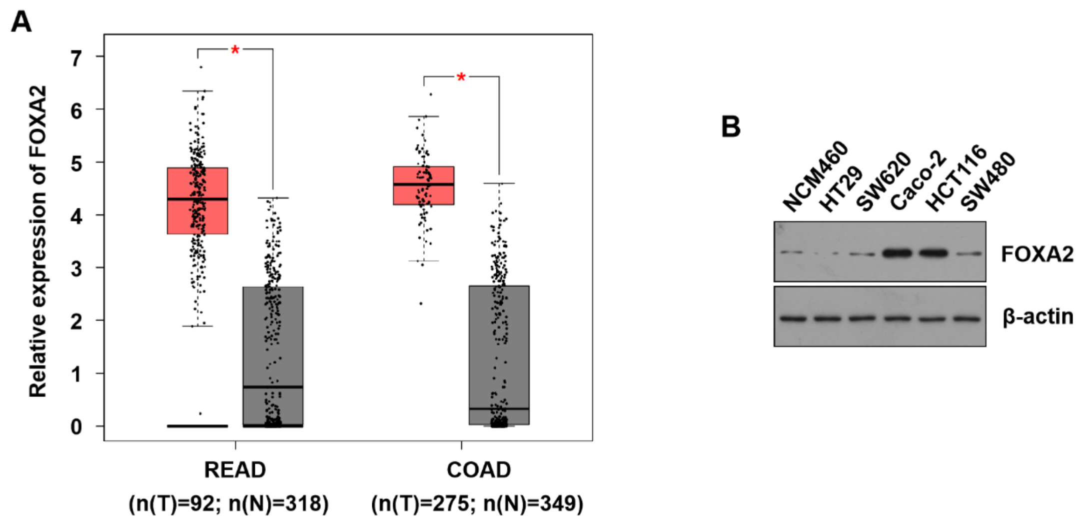

Fig. 1: FOXA2 expression is upregulated in CRC samples and cell lines. A. *FOXA2* mRNA level was analyzed by using Gene Expression Profiling Interactive Analysis (GEPIA) in colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). B. Western blot analysis showing FOXA2 protein levels in normal human colonic epithelial cell line NCM460 and human colorectal cancer cell lines (HT-29, SW620, Caco-2, HCT116, and SW480). β-actin acted as the loading control

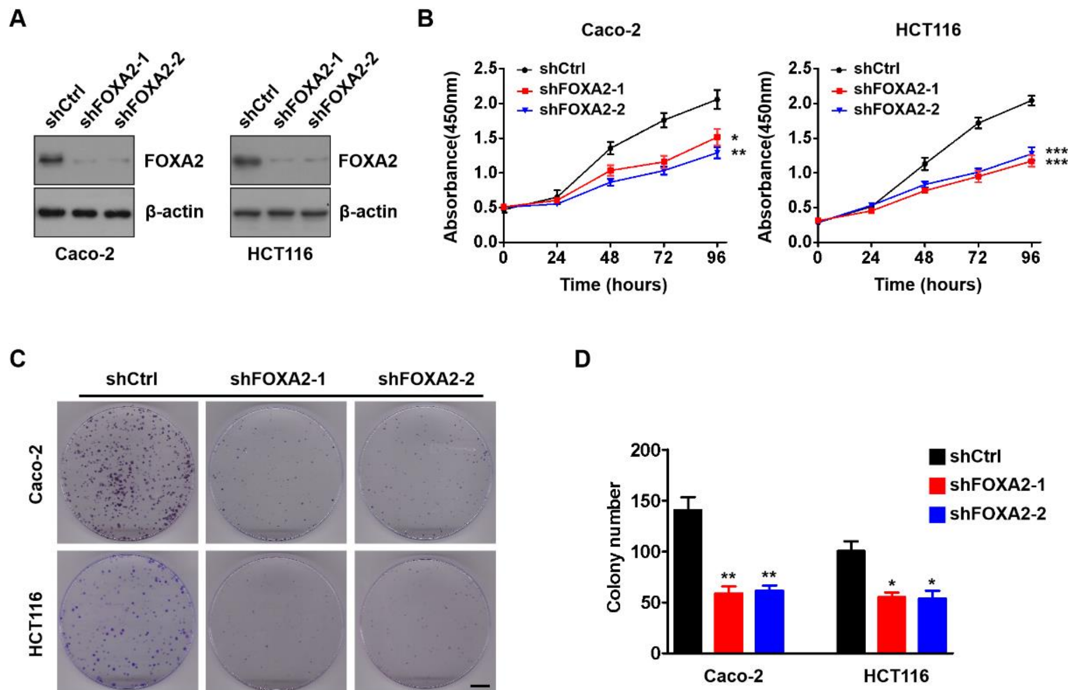

Fig. 2: *FOXA2* knockdown suppresses CRC cells proliferation. A. Western blot analysis showing the silencing efficiency of two independent *FOXA2* shRNA or control shRNA in 2 CRC cell lines. β-Actin served as the loading control. B. CCK-8 assay showing the number of viable cells (Caco-2, left panel and HCT116, right panel) at 24, 48, 72, and 96 hours after the indicated shRNA transfection. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. C, D. The number of colonies formed by Caco-2 and HCT116 cells transduced with *FOXA2* shRNA or control shRNA. $*P < 0.05$, $**P < 0.01$

FOXA2 depletion inhibits CRC cells invasion and migration

Transwell assay showed that *FOXA2* depletion suppressed Caco-2 and HCT116 cell invasion (Fig. 3A, 3B). Wound healing assay suggested that *FOXA2*-depleted Caco-2 cells had less wound coverage 24h after planting compared with control cells, indicating an inhibited migration capacity (Fig. 4A, 4B). Parallel experiments were performed in HCT116 cells, and similar trends were observed (Fig. 4C, 4D).

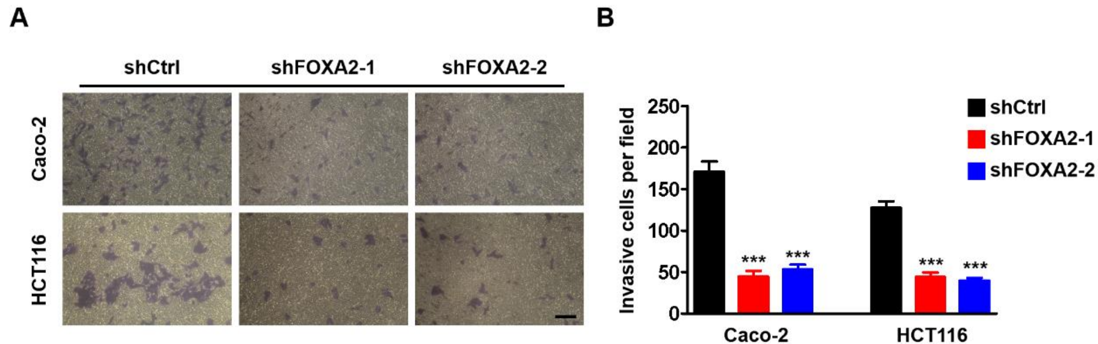

Fig. 3: Depletion of *FOXA2* inhibits CRC cells invasion. A, B. Transwell invasion assay showing the invasion capacity of Caco-2 and HCT116 cells transduced with the indicated shRNA. Quantification of invasion assay was shown (B). ***$P < 0.001$, n = 3 experiments. Scale bar = 200 μm

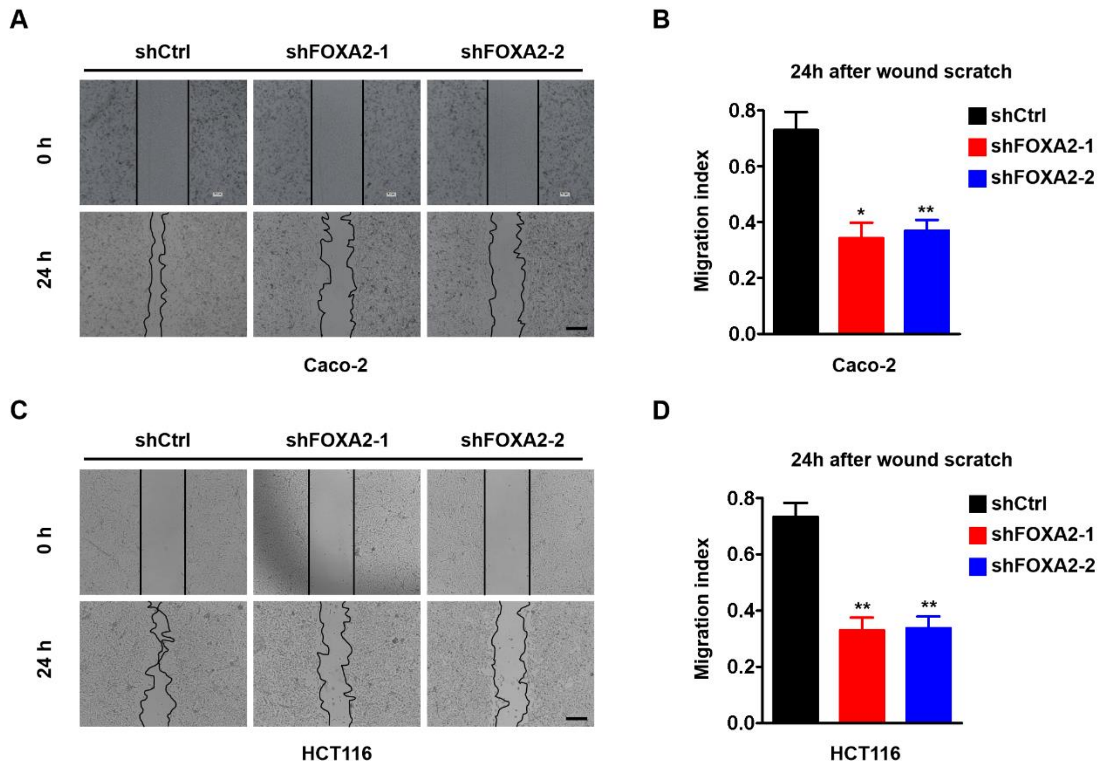

Fig. 4: Depletion of *FOXA2* inhibits CRC cells migration. A-D. Wound coverage of Caco-2 cells (A) and HCT116 cells (C) transduced with *FOXA2* shRNA or control shRNA 24 h after scratching. Quantification of wound healing assay was shown (B, D). *$P < 0.05$, **$P < 0.01$, n = 3 experiments. Scale bar = 200 μm

BAX is a potential target of FOXA2

We noted that *FOXA2* silencing drastically reduced the protein level of BAX, while CITED2 and CADM1 protein levels were not affected (Fig. 5A, 5B). Intriguingly, we barely detected the expression of NR0B2 by Western blot analysis in these two kinds of CRC cells.

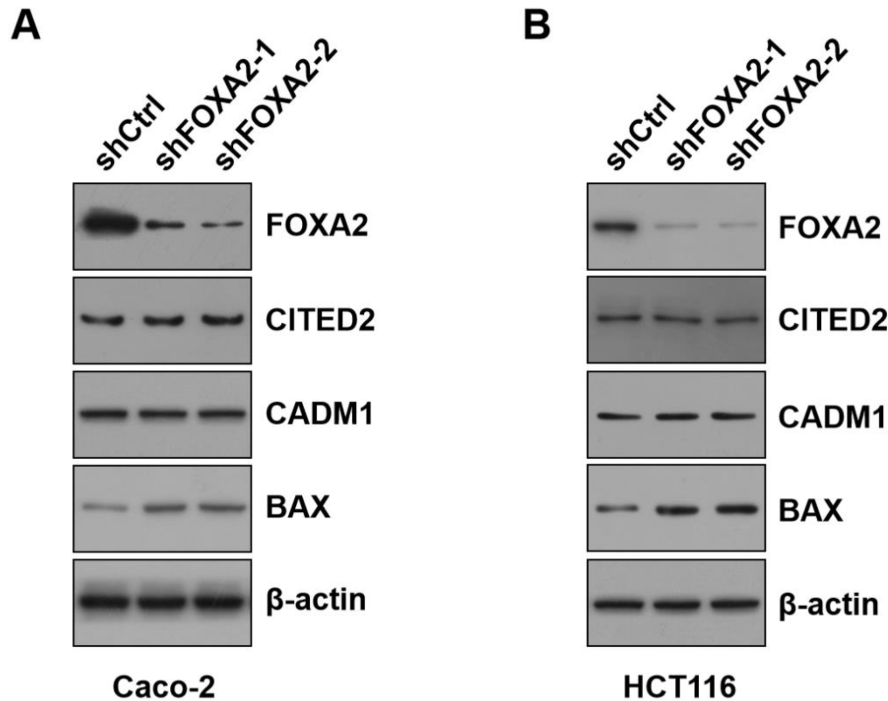

Fig. 5: BAX is a potential target of *FOXA2*. The protein levels of CITED2, CADM1, and BAX were analyzed using Western blot in Caco-2 (A) and HCT116 (B) cells, respectively. β-Actin was used as the loading control

Discussion

FOX family genes are involved in various types of human cancers initiation and development as oncogenes and/or tumor suppressor genes (28). In the present study, our in vitro functional experiments, combined with analysis of clinical samples, which were acquired from the public database, demonstrated that *FOXA2* might be a critical molecule that is responsible for tumor progression in CRCs.

CRC is composed of colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). To investigate the expression level changes of *FOXA2* in CRC, we first extracted and analyzed the mRNA level of *FOXA2* in COAD and READ from Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/). We found that the level of *FOXA2* was substantially upregulated in READ and COAD compared with their corresponding normal tissues. FOXA2 protein levels in CRC cell lines were higher than those in normal human colonic epithelial cells. Thus, these findings unveiled the importance of *FOXA2* in CRC and the possibility of *FOXA2* as a potential tumor-promoting gene.

To our knowledge, cancer is an abnormal cell mass or tumor, except for hematological cancers, shares properties such as uncontrolled cell proliferation and inhibition of cell death (29). The strategy of targeting this kind of uncontrolled proliferation in tumor cells represents a reliable approach to cancer treatment and control. Of note, chemotherapy, which mechanism is aimed to reduce or inhibit the division of rapidly growing cells, has been listed as first-line therapy for advanced cancers and/or as a supplement for

surgical intervention (29). In this study, to elucidate the function of *FOXA2* in CRC, we established two types of *FOXA2*-depleted CRC cell lines with two independent *FOXA2* shRNAs or control shRNA. We provided direct evidence to demonstrate that *FOXA2* knockdown dramatically inhibited the growth of CRC cells, indicating that *FOXA2* was required for CRC cells proliferation. *FOXA2*, as a direct target of miR-200a, promotes hepatocellular carcinoma cell proliferation (30). Although effective inhibitors of *FOXA2* have not been reported, the striking inhibitory effect of *FOXA2* on CRC cells growth strongly suggests that further efforts toward identification of inhibitors of *FOXA2* are fully warranted.

The invasion and migration of tumor cells to adjacent and distant tissues are the major contributors to CRC malignant progression (31, 32). The invasive properties of tumor cells into normal peripheral tissue pose a great challenge to current treatments for CRCs (33). Further, we tried to determine whether loss of *FOXA2* could influence the ability of CRC cells to invade and migrate via transwell and wound healing assays, separately. In our study, we discovered that the invasion and migration ability of *FOXA2*-depleted CRC cells is strongly inhibited compared with control cells, indicating the important role of *FOXA2* in CRC invasion and migration. Notably, *FOXA2* tends to be a double-edged sword in cancer initiation and progression. PHF8 transcriptionally upmodulates *FOXA2* expression and subsequently drives neuroendocrine prostate cancer development (34). *FOXA2* fosters cells proliferation, maintains stem cells self-renewal, and positively correlates with patients' poor clinical outcome in Triple-Negative/Basal-like tumors (15). By contrast, enhanced expression of *FOXA2* was reported to link to decreased lymph node metastasis in oral cancer and prolonged patients' survival time (35). Carman Man-Chung Li et al. also presented evidence that *FOXA2* and the *NK2-related homeobox transcription factor 1* (*Nkx2-1*) synergize with *caudal-related homeobox transcription factor 2* (*Cdx2*) to suppress activation of the metastatic program in lung adenocarcinoma. Our very first focus on the function of *FOXA2* in CRC is of great significance due to the discrepancies in the role of *FOXA2* in different cancers.

Transcription factors *Glu/Asp-rich carboxyterminal domain 2* (*CITED2*), *nuclear receptor subfamily 0, group B, member 2* (*NR0B2*), *cell adhesion molecule 1* (*CADM1*) and *BAX* are identified as putative target of *FOXA2* in non-small cell lung cancer (24). *FOXA2* is recruited and bound to the regions of the *CITED2*, *NR0B2*, *CADM1*, and *BAX* promoters (24). Thus, we wondered whether *FOXA2* also participates in the regulation of the aforementioned proteins in CRC. To test our hypothesis, we knocked down *FOXA2* in Caco-2 and HCT116 cells using two independent *FOXA2* shRNAs. *FOXA2* silencing drastically reduced the protein level of BAX. BAX, a promoter of cell death, is frequently inactivated and/or mutated in cancer cells, which is strongly associated with impediment of apoptosis (36). Moreover, decreased BAX expression is lined to differentiate poorly histological type of CRCs (21). In our study, we found that BAX, one of the potential targets of *FOXA2* (24), was enhanced at protein levels after *FOXA2* silencing in CRC cell lines. Therefore, our findings suggested that *FOXA2* might exert its function via specifically targeting BAX in CRCs.

Conclusion

This study for the first time explored *FOXA2* expression in CRC tissues and cell lines. Further in vitro functional experiments showed that downregulation of *FOXA2* significantly impeded CRC cells growth, invasion and migration. In addition, BAX was identified as a potential downstream target of *FOXA2*. However, lack of in vivo research on the role of *FOXA2* in CRC constitutes a major limitation in this study. Taken together, our study highlights the importance of the *FOXA2*-BAX signaling axis and suggests that targeting this novel pathway may open new therapeutic avenues for CRCs.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB (2019). Colorectal cancer. *The Lancet,* 394:1467-1480.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, 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.
3. Siegel RL, Miller KD, Jemal A (2019). Cancer statistics, 2019. *CA Cancer J Clin,* 69:7-34.
4. Katoh M, Katoh M (2004). Human FOX gene family (Review). *Int J Oncol,* 25:1495-500.
5. Ploton M, Mazuy C, Gheeraert C, et al (2018). The nuclear bile acid receptor FXR is a PKA- and FOXA2-sensitive activator of fasting hepatic gluconeogenesis. *J Hepatol,* 69:1099-1109.
6. Dhakal P, Kelleher AM, Behura SK, Spencer TE (2020). Sexually dimorphic effects of forkhead box a2 (FOXA2) and uterine glands on decidualization and fetoplacental development. *Proc Natl Acad Sci U S A,* 117:23952-23959.
7. Bochkis IM, Rubins NE, White P, Furth EE, Friedman JR, Kaestner KH (2008). Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. *Nat Med,* 14:828-36.
8. Li CM, Gocheva V, Oudin MJ, et al (2015). Foxa2 and Cdx2 cooperate with Nkx2-1 to inhibit lung adenocarcinoma metastasis. *Genes Dev,* 29:1850-62.
9. Tang Y, Shu G, Yuan X, Jing N, Song J (2011). FOXA2 functions as a suppressor of tumor metastasis by inhibition of epithelial-to-mesenchymal transition in human lung cancers. *Cell Res,* 21:316-26.
10. Chu GC, Zhau HE, Wang R, et al (2014). RANK- and c-Met-mediated signal network promotes prostate cancer metastatic colonization. *Endocr Relat Cancer,* 21:311-26.
11. Ozaki T, Matsubara T, Seo D, et al (2012). Thyroid regeneration: characterization of clear cells after partial thyroidectomy. *Endocrinology,* 153:2514-25.
12. Zhang Z, Yang C, Gao W, et al (2015). FOXA2 attenuates the epithelial to mesenchymal transition by regulating the transcription of E-cadherin and ZEB2 in human breast cancer. *Cancer Lett,* 361:240-50.
13. Cao L, Gibson JD, Miyamoto S, et al (2011). Intestinal lineage commitment of embryonic stem cells. *Differentiation,* 81:1-10.
14. Kaestner KH (2005). The making of the liver: developmental competence in foregut endoderm and induction of the hepatogenic program. *Cell Cycle,* 4:1146-8.
15. Perez-Balaguer A, Ortiz-Martinez F, Garcia-Martinez A, et al (2015). FOXA2 mRNA expression is associated with relapse in patients with Triple-Negative/Basal-like breast carcinoma. *Breast Cancer Res Treat,* 153:465-74.
16. Song Y, Washington MK, Crawford HC (2010). Loss of FOXA1/2 is essential for the epithelial-to-mesenchymal transition in pancreatic cancer. *Cancer Res,* 70:2115-25.
17. Kaestner KH (2010). The FoxA factors in organogenesis and differentiation. *Curr Opin Genet Dev,* 20:527-32.
18. Friedman JR, Kaestner KH (2006). The Foxa family of transcription factors in development and metabolism. *Cell Mol Life Sci,* 63:2317-28.
19. Reyna DE, Garner TP, Lopez A, et al (2017). Direct Activation of BAX by BTSA1 Overcomes Apoptosis Resistance in Acute Myeloid Leukemia. *Cancer Cell,* 32:490-505 e10.

20. Liu Z, Ding Y, Ye N, Wild C, Chen H, Zhou J (2016). Direct Activation of Bax Protein for Cancer Therapy. *Med Res Rev,* 36:313-41.
21. Jansson A, Sun XF (2002). Bax expression decreases significantly from primary tumor to metastasis in colorectal cancer. *J Clin Oncol,* 20:811-6.
22. Dong H, Wang W, Chen R, et al (2018). Exosome-mediated transfer of lncRNASNHG14 promotes trastuzumab chemoresistance in breast cancer. *Int J Oncol,* 53:1013-1026.
23. Liu X, Dong J, Cai W, Pan Y, Li R, Li B (2017). The Effect of Thymoquinone on Apoptosis of SK-OV-3 Ovarian Cancer Cell by Regulation of Bcl-2 and Bax. *Int J Gynecol Cancer,* 27:1596-1601.
24. Jang SM, An JH, Kim CH, Kim JW, Choi KH (2015). Transcription factor FOXA2-centered transcriptional regulation network in non-small cell lung cancer. *Biochem Biophys Res Commun,* 463:961-7.
25. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res,* 45:W98-W102.
26. Pompsch M, Vogel J, Classen F, et al (2018). The presumed MTH1-inhibitor TH588 sensitizes colorectal carcinoma cells to ionizing radiation in hypoxia. *BMC Cancer,* 18:1190.
27. Hirano S (2012). Western blot analysis. *Methods Mol Biol,* 926:87-97.
28. Katoh M, Igarashi M, Fukuda H, Nakagama H, Katoh M (2013). Cancer genetics and genomics of human FOX family genes. *Cancer Lett,* 328:198-206.
29. Perez-Herrero E, Fernandez-Medarde A (2015). Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *Eur J Pharm Biopharm,* 93:52-79.
30. Chen SY, Ma DN, Chen QD, et al (2017). MicroRNA-200a inhibits cell growth and metastasis by targeting Foxa2 in hepatocellular carcinoma. *J Cancer,* 8:617-625.
31. Rokavec M, Oner MG, Li H, et al (2014). IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest,* 124:1853-67.
32. Lan J, Sun L, Xu F, et al (2019). M2 Macrophage-Derived Exosomes Promote Cell Migration and Invasion in Colon Cancer. *Cancer Res,* 79:146-158.
33. Friedl P, Wolf K (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer,* 3:362-74.
34. Liu Q, Pang J, Wang LA, et al (2021). Histone demethylase PHF8 drives neuroendocrine prostate cancer progression by epigenetically upregulating FOXA2. *J Pathol,* 253:106-118.
35. Bow YD, Wang YY, Chen YK, et al (2020). Silencing of FOXA2 decreases E-cadherin expression and is associated with lymph node metastasis in oral cancer. *Oral Dis,* 26:756-765.
36. Pryczynicz A, Gryko M, Niewiarowska K, Cepowicz D, Ustymowicz M, Kemona A, Guzinska-Ustymowicz K (2014). Bax protein may influence the invasion of colorectal cancer. *World J Gastroenterol,* 20:1305-10.