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Research article

FOXM1c is the predominant FOXM1 isoform expressed in cholangiocarcinoma that associated with metastatic potential and poor prognosis of patients

Nathakan Klinhom-on [a], Wunchana Seubwai [b,d], Kanlayanee Sawanyawisuth [a,d], Worachart Lert-itthiporn [a,d], Sakda Waraasawapati [c,d], Marutpong Detarya [a], Sopit Wongkham [a,d,*]

[a] Department of Biochemistry, Faculty of Medicine, Khon Kaen University, 40002, Thailand
[b] Department of Forensic Medicine, Faculty of Medicine, Khon Kaen University, 40002, Thailand
[c] Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[d] Cholangiocarcinoma Research Institute, Khon Kaen University, 40002, Thailand

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ABSTRACT

Forkhead box M1 (FOXM1) is a transcriptional factor which plays an important role in oncogenesis. Four FOXM1 isoforms, FOXM1a, FOXM1b, FOXM1c and FOXM1d, are known so far. Different FOXM1 isoforms influence progression of cancer in different cancer types. In this study, the FOXM1c isoform and its impact in cholangiocarcinoma (CCA) was identified. FOXM1c was found to be the predominant isoform in patient-CCA tissues and cell lines. Detection of FOXM1c expression in CCA tissues reflected the worse prognosis of the patients, namely the advanced stage and shorter survival. Suppression of FOXM1 expression using siRNA considerably reduced migration and invasion abilities of CCA cell lines. RNA sequencing analysis revealed claudin-1 as a target of FOXM1. FOXM1 exhibited a negative correlation with claudin-1 expression which was demonstrated in patient CCA tissues and cell lines. FOXM1 may be a potential target for therapeutic treatment of the metastatic CCA.

1. Introduction

Cholangiocarcinoma (CCA) is a bile duct malignancy that originates in both intra- and extra-hepatic ducts. The incidence of CCA is high in northeastern Thailand and is now increasing worldwide [1]. In general, CCA has no signs or symptoms during development, leading to difficulties in early and late diagnoses when CCA has already developed into the metastatic stage [2]. CCA possesses a high mortality rate according to high metastasis and ineffective treatment. Understanding the molecular mechanisms of metastasis may reveal a novel target treatment for CCA.

Forkhead box M1 (FOXM1) protein is a transcriptional factor in the Forkhead box protein family which is necessary in many biological processes [3]. The oncogenic properties of FOXM1 have been related to all hallmarks of cancer, e.g., cell proliferation, migration and invasion [4]. FOXM1 gene contains 10 exons, of which 2 exons, Va and VIIa, are alternatively spliced, resulting in the formation of four isoforms: FOXM1a, FOXM1b, FOXM1c and FOXM1d (Supplementary Figure 1). Due to the alternative splicing, FOXM1b and FOXM1c are transcriptionally active and locate predominantly in the nucleus, while FOXM1a and FOXM1d are transcriptionally inactive and locate exclusively in the cytoplasm [5, 6]. The impact of FOXM1 isoforms on cancer progression has been collectively reported as follows: Each FOXM1 isoform is expressed as the major isoform in different types of cancer. For instance, FOXM1b is the major isoform reported in cancer cell lines from lung, breast, cervix, ovary, and myeloid leukemia [7], as well as hepatocellular carcinoma, of which FOXM1b has been shown to be associated with development and metastasis [8]. On the other hand, FOXM1c was the predominant isoform in pancreatic cancer cell lines and shown to be required for tumor growth and metastasis in an animal model [9].

Using laser microbeam microdissection and cDNA microarray analysis, FOXM1 was found to be upregulated in more than 90% of tumor tissues from CCA patients [10]. The FOXM1 isoforms and its significance in CCA, however, have not been investigated. In the present study, the expression of FOXM1 isoforms was determined in CCA tissues from Thai patients and CCA cell lines using conventional polymerase chain reactions (PCR) and real-time PCR techniques. The correlation between the

* Corresponding author.
E-mail address: sopit@kku.ac.th (S. Wongkham).

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major FOXM1 isoforms and clinicopathological features of CCA patients was analyzed. In addition, the impact of FOXM1 on progression of CCA cells and the mechanisms underlying the FOXM1 action were revealed.

2. Materials and methods

2.1. Patient tissues

The histologically proven CCA tissues (n = 40) and the corresponding normal adjacent tissues (n = 13) were obtained from the Biobank of the Cholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Thailand. Informed consent was obtained from each subject and the study protocol was approved by the Human Research Ethics Committee, Khon Kaen University (HE621291).

2.2. CCA cell lines

CCA cell lines; KKU-055, KKU-100, KKU-213A, and KKU-213B established from the primary tumors of Thai CCA patients as previously described [11, 12] were obtained from the Japanese Collection of Research Bioresource (JCBR) Cell Bank, Osaka, Japan. KKU-055 and KKU-100 were established from poorly differentiated type-CCA tissues, whereas KKU-213A and KKU-213B cell lines were established from a mixed type of papillary and non-papillary CCA and possessed high metastatic characteristics compared with KKU-055 and KKU-100. All cell lines were cultured in Dulbecco's modified Eagle's medium with high glucose (DMEM; 12800-058, Gibco/Invitrogen, Calsbald, CA) containing 10% fetal bovine serum (Gibco/Invitrogen) and a 1% antibiotic-antimycotic (Penicillin/Streptomycin/Amphotericin B) additive (Gibco/Invitrogen), in a humidified incubator at 37 °C and 5% CO_2.

2.3. RNA extraction and cDNA synthesis

Total RNA from tissues and cell lines were extracted in Trizol® reagent (Invitrogen) and reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied biosystems, Carlsbad, CA), according to the manufacturer's instructions.

2.4. Conventional PCR

PCR was performed using cDNA samples (50 ng) and the i-taq™ plus DNA Polymerase kit (Intron Biotechnology, Korea) with the specific primers. The primers of FOXM1a, FOXM1b, and FOXM1c were as described previously [9]. The PCR products were subjected to an agarose gel electrophoresis and visualized with ethidium bromide staining using ImageQuant LAS500 (GE Healthcare, Buckinghamshire, UK).

2.5. Real-time PCR

Real-time PCR analysis for FOXM1 isoform expression was performed using the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany), with SYBR Green Master Mix (Roche Diagnostics). The PCR master mixtures contained 0.5 μM of forward and reverse primers, 1x SYBR Green Master Mix, and 50 ng of cDNA in a final volume of 10 μl. Each cDNA sample was run in triplicate for the target and internal control genes.

2.6. Transient knockdown of FOXM1 by siRNA

The small interfering RNA (siRNA) sequence targeting FOXM1 (siFOXM1) was siFOXM1 SMARTpool: ON-TARGETplus FOXM1 siRNA, L009762-00-0010 (Dharmacon, CO, USA). Transfection of siRNAs into cell lines was performed using the Lipofectamine 2000 transfection reagents (Invitrogen). Cells were transfected with siRNA or scramble siControl (1027310, Qiagen, Chatsworth, CA) at 50 ρmole for 48 h prior to subsequent analysis.

2.7. Western blot analysis

Cell lysis, SDS-polyacrylamide gel electrophoresis and Western blottings were performed as previously described [13]. The immunoreaction was developed using the ECL prime Western blot detection system (GE Healthcare). The images of ECL signals were taken and analyzed using the Amersham Imager600 (AI600) and AI600 analysis software (GE Healthcare). The specific primary antibodies for Western blot analysis were 1:10,000 anti-β-actin (A5441, Sigma Aldrich, St. Louis, MO), 1:200 FOXM1 (sc-502 and sc-376471; Santa Cruz Biotechnology, Santa Cruz, CA), and 1:1,000 claudin-1 (13255S; Cell Signaling Technology; Denvers, MA).

2.8. Migration and invasion assays

Migration and invasion assays were performed using a Boyden chamber assay with an 8 μm pore size insert (Corning Incorporated, Corning, NY), as previously described [13]. Briefly, 4×10^4 CCA cells in a serum-free DMEM medium were allowed to migrate or invade, 6 h for KKU-213A and 24 h for KKU-213B. After the incubation period, the migrated or invaded cells underneath the filter were fixed with paraformaldehyde and stained with Sulforhodamine B. The stained cells were observed under a microscope and counted.

2.9. RNA-sequencing and bioinformatics analysis

RNA-sequencings of three biological replicates each of KKU-213A cells with siRNA or siControl treatment for 48 h were performed at NovogeneAIT, Singapore. The RNA purity and integrity were determined using nanodrop/agarose gel electrophoresis, and the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA). The library preparation was constructed using a NEBNext® Ultra™ RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) using the manufacturer's protocol. After cDNA size selection (150–200 base pairs) and PCR enrichment, the sequencing was carried out on an Illumina system.

The raw data from sequencing was processed for quality, followed by mapping the clean reads to the reference genome using STAR software before downstream analysis. The gene expression level was estimated as fragments per kilobase of the transcription sequence per millions base pairs sequenced (FPKM), and the DESeq2 R package [14] was used to analyze the differentially expressed genes of the two conditions. Pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) [15].

2.10. Statistical analysis

SPSS statistics 23.0 software (SPSS, Inc.; Chicago, IL) and GraphPad Prism® 5.0 software (GraphPad Software, Inc., La Jolla, CA) were used for statistical analysis. The Kaplan-Meier survival plot with the log-rank test were used for survival analysis. The Cox regression model with a subsequent stepwise backwards was used for multivariate analysis. The clinicopathological features of patients were compared using the chi-square test. Correlograms and Pearson analysis were conducted for the gene correlation using R-studio. The significance of the data was determined using Student's t-test (two-tailed) and P values < 0.05 were considered significant.

3. Results

3.1. FOXM1c was the major isoform aberrantly expressed in CCA tissues

Expression levels of FOXM1 mRNA in patient CCA tissues and adjacent normal tissues were first analyzed using the data obtained from Gene Expression Profiling Interactive Analysis (GEPIA1; gepia.cancer-pku.cn). CCA tissues (n = 36) exhibited significantly higher FOXM1 mRNA levels than those of adjacent normal tissues (n = 9) (Figure 1A).

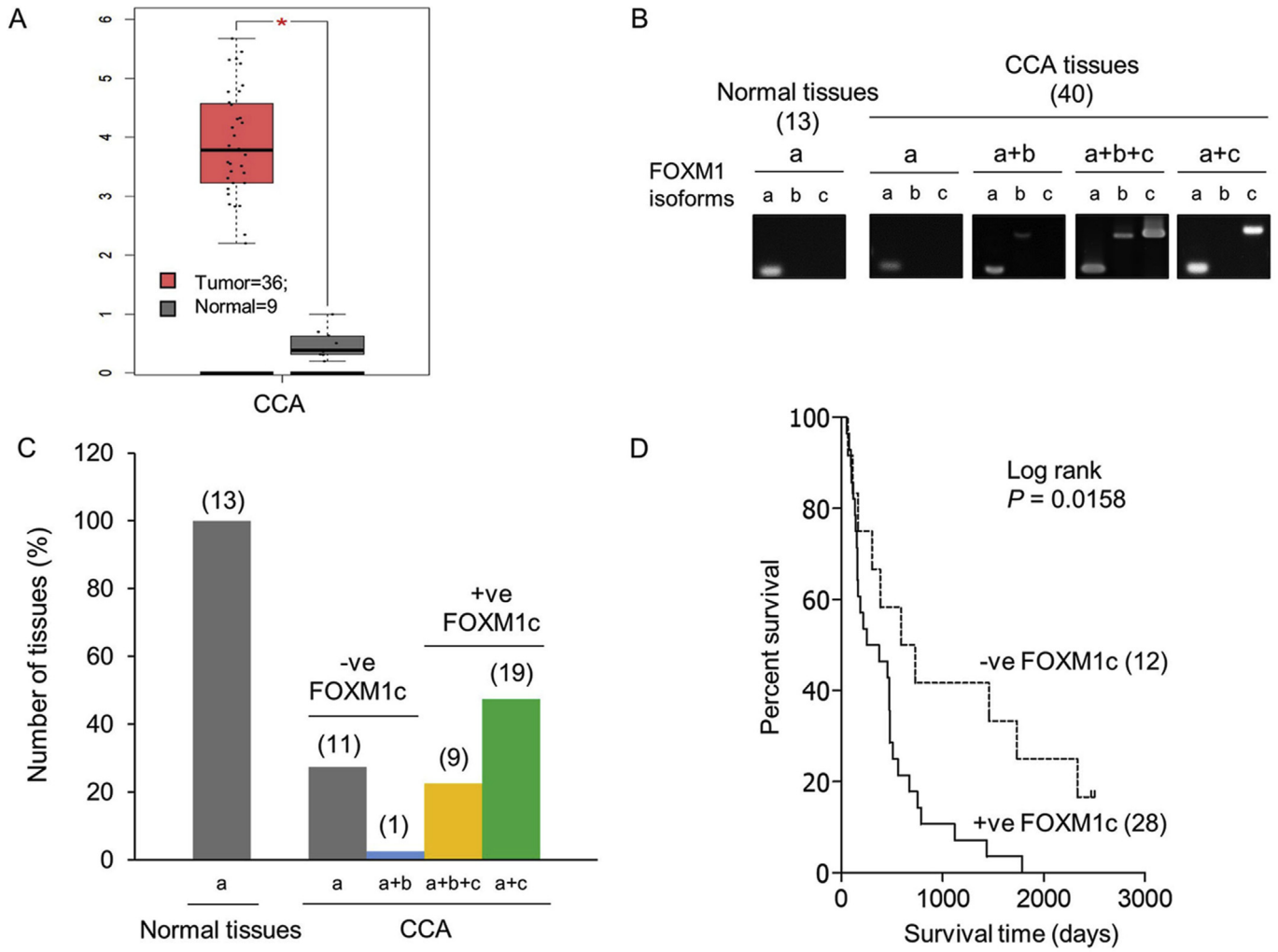

Figure 1. FOXM1c is the major isoform in human CCA tissues. (A) Distribution of FOXM1 expression in CCA tissues compared with its counterpart normal tissues, data from GEPIA1 webserver (gepia.cancer-pku.cn). *, P < 0.05. (B) Four expression patterns of FOXM1 isoforms found in CCA tissues. The mRNAs of FOXM1 isoforms were determined in patient CCA tissues and the normal adjacent tissues using endpoint PCR. (C) Distribution of FOXM1 isoform patterns. (D) Kaplan-Meir survival curves with the log-rank test for negative (-ve) and positive (+ve) FOXM1c expressing cases. Numbers in the parentheses are number of patients.

The expressions of FOXM1 isoforms in 40 CCA tumors and 13 corresponding para-tumor tissues were determined using conventional PCR and agarose gel electrophoresis. As shown in Figure 1B, only FOXM1a isoform was detected in all 13 normal tissues. In contrast, FOXM1a, and FOXM1a with FOXM1b and/or FOXM1c were observed in CCA tissues, generating four patterns of FOXM1 expression: 1) FOXM1a alone (a), 2) FOXM1a and FOXM1b (a+b), 3) FOXM1a, FOXM1b and FOXM1c (a+b+c), and 4) FOXM1a and FOXM1c (a+c). According to the semi-

Table 1. Associations between FOXM1c expression and clinicopathological characteristics of CCA patients (n = 40).

Variables		n	FOXM1c		*P*-value
			Negative	Positive	
Gender	Female	17	3	14	
	Male	23	9	14	0.179
Age (year)	<58	20	7	13	
	≥58	20	5	15	0.731
Tumor size (cm)	<5	11	4	7	
	≥5	29	8	21	0.589
Histological type	Papillary	17	5	12	
	Non papillary	23	7	16	1.000
Lymph node metastasis (n = 33)	Negative	11	4	18	
	Positive	22	4	7	0.251
Stage (n = 38)	I–III	17	8	9	
	IV	21	2	19	0.023*

*, $P < 0.05$.

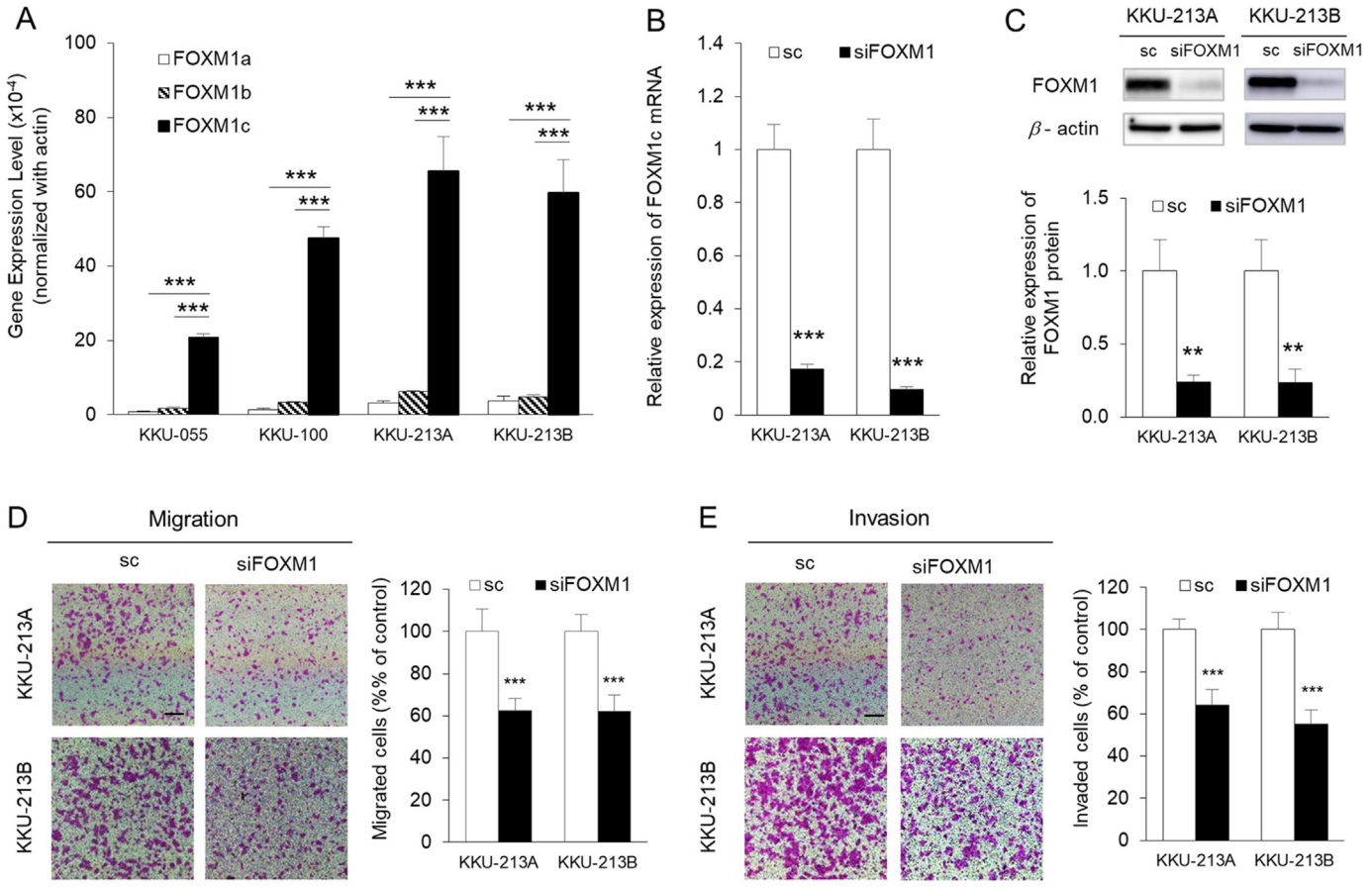

Figure 2. FOXM1 is associated with progression of CCA cells. (A) Expression levels of FOXM1a, FOXM1b, and FOXM1c mRNA were determined in 4 CCA cell lines using real-time PCR. siFOXM1 treatment significantly suppressed expression levels of FOXM1 in both (B) mRNA and (C) protein. (D) Migration and invasion properties of siFOXM1 treated cells were decreased compared with the scramble control cells (sc). The experiments were done in triplicate with 3 independent biological samples. The data are means ± SD; (A) and (B) are a representative; (C–E) are from 3 independent experiments. Scale bar = 500 μm. **, $P < 0.01$; ***, $P < 0.001$.

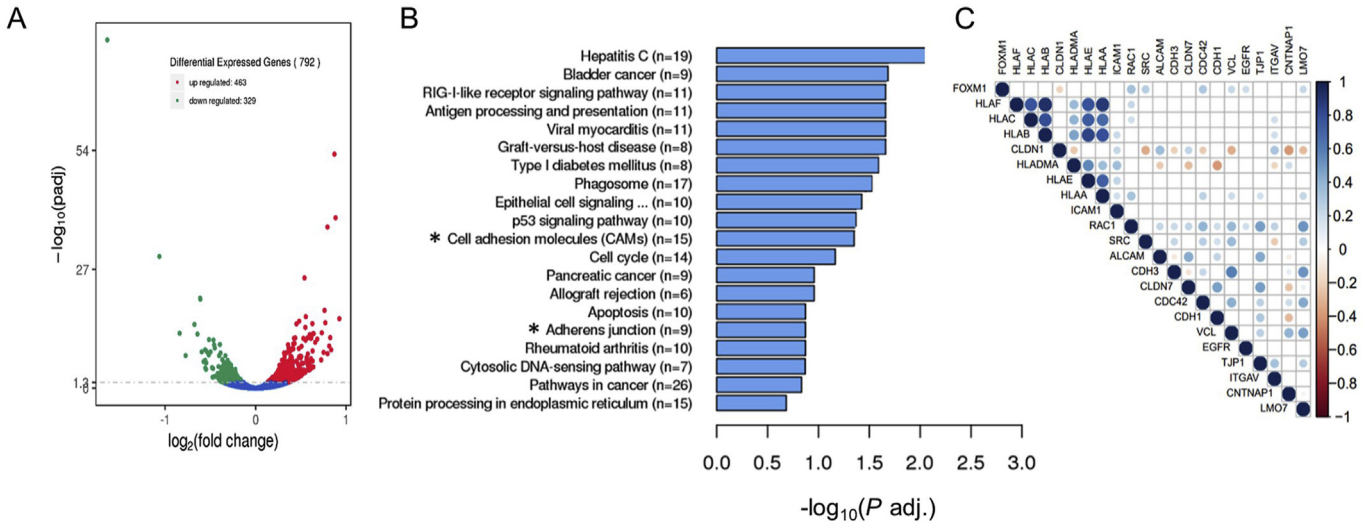

Figure 3. Bioinformatic analysis of FOXM1 and FOXM1related genes in patient CCA tissues. Gene expression profiling of siFOXM1 vs. scramble treated KKU-213A cells were determined using RNA-seq. (A) Volcano plot visualizes the significantly differential expressed genes with Padj<0.05. (B) The top 20 significantly enriched KEGG pathways with corrected P-values<0.05. (C) Correlogram illustrates the cross-correlations of FOXM1 and metastasis-associated genes in patient CCA tissues retrieved from the GSE89749 dataset [17]. Circles indicate correlations with $P < 0.05$; size of circle represents correlation co-efficiency (R value); blue and red colors represent the positive and negative correlations. *P*adj = *P* adjusted; *pathways involved with metastasis.

Table 2. Genes related to adhesion molecules and correlated with FOXM1 expression based on transcriptome analysis.

Gene symbols	Gene names	Fold change	*p*-adj. value
CLDN1	Claudin-1	1.35	2.17×10^{-3}
RAC1	Ras-related C3 botulinum toxin substrate 1	1.19	1.39×10^{-4}
SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase	1.14	2.01×10^{-2}
CDC42	Cell Division Cycle 42	0.90	3.04×10^{-2}
VCL	Vinculin	0.89	1.04×10^{-2}
EGFR	Epidermal Growth Factor Receptor	0.87	2.67×10^{-2}
LMO7	LIM Domain 7	0.80	4.00×10^{-3}

p-adj. = adjusted *P* value.

quantitative analysis of FOXM1 expression (Figure 1B), FOXM1c was found to be the dominant isoform and was the most frequently observed isoform in CCA tissues, accounting for 70% (28/40) (Figure 1C). Hence, FOXM1c was selected for further investigation in the subsequent study.

3.2. FOXM1c isoform was a prognosis marker of worse progression of CCA

To investigate the clinical significance of FOXM1c in CCA, the univariate analysis of FOXM1c expression and clinico-pathological features of CCA patients were determined. As shown in Table 1, FOXM1c expression was significantly related with the tumor stage ($P = 0.023$). CCA patients with metastatic stage (stage IV) exhibited FOXM1c expression more frequently than those in stages I-III. The association between FOXM1c expression and survival of CCA patients was demonstrated using Kaplan-Meier analysis. As shown in Figure 1D, patients with positive FOXM1c had significantly shorter survival times than those with negative FOXM1c ($P = 0.016$). The median survival time of the patients with positive FOXM1c was 252 ± 177 days and those with negative FOXM1c was 591 ± 298 days. The univariate analysis using the Cox regression model indicated the influence of FOXM1c expression on survival of CCA patients ($P = 0.019$) with a hazard ratio of 2.568 (95% Confidence interval, CI; 1.166–5.657). Moreover, the multivariate analysis implied that FOXM1c was an independent factor for short survival of CCA patients ($P = 0.023$) with a hazard ratio 4.015, regardless of gender, age, tumor size, histological type and lymph node metastasis (Supplementary Table 1). These results suggest FOXM1c as a prognosis marker associated with worse progression and survival of CCA patients.

3.3. FOXM1c isoform played a role in progressive phenotypes of CCA cell lines

The association of FOXM1 isoforms with progression of CCA cells were next examined. FOXM1 isoforms were investigated in 4 CCA cell lines, KKU-055, KKU-100, KKU-213A, and KKU-213B, using real-time PCR analysis. FOXM1c was shown to be the major isoform in all CCA cell lines tested (Figure 2A). KKU-213A and KKU-213B with high expression levels of FOXM1c were selected for the subsequent studies. Because of the high homology of mRNA sequences of FOXM1a and FOXM1c (approximately 97%) limited the siRNA designed for FOXM1c, thus the siRNA specifically to total FOXM1 isoforms (siFOXM1 SMARTpool, Dharmacon) was used [16]. The siRNA could suppress approximately 80% of the mRNA of all FOXM1 isoform studied (Supplementary Figure 2). Compared to the scramble treated cells, expression levels of FOXM1c mRNA and protein were significantly reduced 80–90% in KKU-213A and KKU-213B cells treated with siFOXM1 for 24 h, as revealed by real-time PCR (Figure 2B) and Western blot analysis (Figure 2C).

The effects of FOXM1c on progression of CCA cells, namely migration and invasion, were next determined. Suppression of FOXM1c expression using siFOXM1 for 24 h significantly decreased the migration of KKU-213A and KKU-213B to 62.4 ± 5.8% and 62.2 ± 7.7% (Figure 2D); and invasion to 64.2 ± 7.2% and 55.1 ± 6.8% (Figure 2E). These results suggest the important roles of FOXM1c in progression of CCA.

3.4. RNA sequencing analysis revealed the association of FOXM1 with innate immunity, cancer, and adhesion

To reveal the FOXM1 regulated genes that were involved in CCA progression, the whole transcriptome of siFOXM1-treated vs. sc-treated KKU-213A cells were analyzed using high-throughput RNA sequencing. There were 792 genes differentially expressed between sc- and siFOXM1 treated cells, 463 upregulated and 329 down regulated as shown by volcano plot (Figure 3A). Using KEGG pathway enrichment analysis, several significant pathways associated with FOXM1 expression in CCA cells were revealed, e.g., innate immunity, cancers and adhesion (Figure 3B). As in this study, FOXM1 was significantly related to migration and invasion, therefore the 21 genes included in the pathways of cell adhesion molecules and adherent junctions were further analyzed. The correlations of these 21 genes with FOXM1 expression were analyzed in a correlogram using the microarray expression profile of patient CCA tissues extracted from the GEO database, GSE89749 [17]. As shown in Figure 3C, 7 of 21 genes were significantly correlated with FOXM1 expression (Table 2).

3.5. Claudin-1 exhibited a negative correlation with FOXM1

Claudin-1 was the highest differentially expressed gene related to FOXM1 suppression, and significantly correlated with FOXM1 as well as several FOXM1 related genes (Figure 3C). In addition, the negative correlation between claudin-1 and FOXM1 expression was demonstrated in the siFOXM1 treated cells. Suppression of FOXM1 expression increased claudin-1 expression for approximately 1.35-fold (Table 2). The positive correlation between FOXM1 and other differentially expressed genes, e.g., RAC1 and SRC, however, could not be demonstrated in the siFOXM1 treated cells (Figure 3C and Table 2). Therefore, claudin-1 was selected to validate its expression in relation to FOXM1. To demonstrate whether claudin-1 is negatively correlated with FOXM1, the expression levels of claudin-1 mRNA and protein were determined in sc- and siFOXM1-treated cells using real-time PCR and Western blot. After knockdown FOXM1, the expression levels of claudin-1 mRNA and protein were significantly increased in siFOXM1-treated cells (Figure 4A, B). To further evaluate whether the negative association of FOXM1 and claudin-1 expression was found in tumor tissues from CCA patients, the association between FOXM1 and claudin-1 was analyzed using microarray expression data extracted from the GEO database; GSE89749 [17]. As shown in Figure 4C, a negative correlation between FOXM1 and claudin-1 expression was confirmed in patient CCA tissues.

4. Discussions

In this study, it has been shown for the first time that FOXM1c was the major isoform that was aberrantly expressed in patient CCA tissues and cell lines. Expression of FOXM1c in CCA tissues was significantly related with the metastatic stage and short survival of CCA patients. In addition,

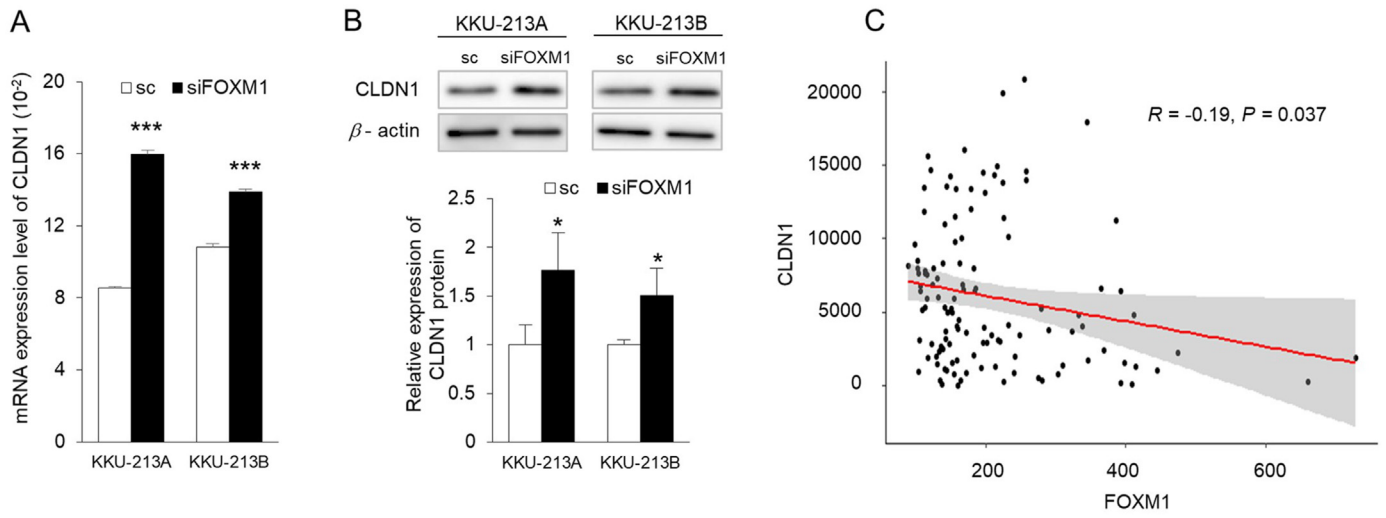

Figure 4. Claudin-1 expression is negatively correlated with FOXM1 expression. Effect of FOXM1 on claudin-1 expression was verified in CCA cells. Knock down of FOXM1 expression using siFOXM1 significantly increased, (A) claudin-1 mRNA and (B) claudin-1 protein, compared with the scramble control cells (sc). (C) The negative correlation of FOXM1 and claudin-1 expression was demonstrated in patient CCA tissues (GSE89749 dataset [17]). The experiments were done in triplicates with 3 independent biological samples. The data are means ± SD; (A) is a representative; (B) is from 3 independent experiments. *, $P < 0.05$; ***, $P < 0.001$ vs. control (sc).

FOXM1c expression possessed a significant role in the migration and invasion abilities of CCA cell lines. The RNA sequencing, real-time PCR and Western blot analyses revealed a negative correlation between expression of claudin-1 and FOXM1.

FOXM1 is a well-known transcriptional factor involved in the biological process of both normal and cancer cells. The activity of FOXM1 is required during embryonic and fetal development and is then reduced in the differentiated adult cells [18]. FOXM1 expression, however, can be activated for proliferation in tissue homeostasis and regeneration [19, 20]. Deregulation of FOXM1 is, therefore, shown to play significant roles in tumorigenesis and progression of cancer.

Four FOXM1 isoforms; FOXM1a, FOXM1b, FOXM1c and FOXM1d are reported so far. Specific FOXM1 isoforms were found predominantly in different types of cancer. Upregulation of FOXM1 was found in human CCA tissues compared to adjacent normal tissues obtained from GEPIA1 (gepia.cancer-pku.cn) and therefore supports the findings of cDNA microarray analyses reported by Jinawath et al. [10]. Determination of FOXM1 isoforms in 40 CCA tissues in comparison with 13 adjacent normal tissues in the present study revealed that FOXM1a was constitutively expressed in both tumor and normal tissues, whereas FOXM1b and FOXM1c were aberrantly expressed only in CCA tissues. FOXM1c was identified as the major isoform in CCA as approximately 70% of patient tissues exhibited this isoform with high expression level. The implication of worse prognosis of FOXM1c expression in CCA patients was drawn by the facts that: 1) FOXM1c expression was related with the metastatic stages of the tumors, 2) patients with FOXM1c expression in tumor tissues had significantly shorter survival than those with a negative FOXM1c, and 3) FOXM1c expression was an independent worse prognosis factor of CCA. These findings strengthened and confirmed the immunohistochemical study reported by Liu et al. [21] that FOXM1 was upregulated in intrahepatic CCA tissues and was correlated with a worse prognosis, e.g., multiple tumor nodules, increased tumor size, lymph node metastasis and advanced TNM stage. The independent prognostic factor of FOXM1 for overall survival was also observed in the same study.

FOXM1 exhibits several oncogenic properties related to all hallmarks of cancer, e.g., cell proliferation, migration and invasion [4]. The association of FOXM1c with metastatic tumors and short survival of patients led to the hypothesis that FOXM1c may play roles in progression of CCA. FOXM1c was also predominantly expressed in CCA cell lines, approximately 10-20-fold higher than FOXM1a and FOXM1b. Silencing of FOXM1 using siFOXM1 could significantly decrease FOXM1c expression to approximately 10–20% of the control cells. Suppression of FOXM1c expression remarkably decreased migration and invasion of two CCA cell lines, KKU-213A and KKU-213B. These findings agreed with those reported in pancreatic cancer, in which FOXM1c was also found as the predominant isoform [9].

Despite the crucial roles of FOXM1 plays in metastasis, the molecular underlayment of the FOXM1-associated CCA progression has not been elucidated. The RNA-sequencing constructed from FOXM1-knockdown vs. the scramble control cells, together with the KEGG pathway enrichment analysis revealed several cancer-associated pathways. Of these, claudin-1, the highest differential gene involved in cell adhesion molecules/adherent junction pathways found from the analysis was further validated. Claudin-1 was correlated with FOXM1 and many genes related to migration and invasion. The negative correlation of FOXM1 and claudin-1 suggested by correlogram analysis was demonstrated in CCA cell lines. Suppression of FOXM1c using siFOXM1 significantly increased the expression level of claudin-1 in both the mRNA and protein (Figure 4A, B). Moreover, the negative connection of FOXM1 and claudin-1 expression could be demonstrated in patient CCA tissues (Figure 4C; GSE89749; [17]). The mechanism by which FOXM1 negatively regulates claudin-1 expression remains unclear and should be further explored.

Claudin-1 (CLDN1) is a member of tight junction proteins that are constituents in cell-cell adhesion and function as a barrier in normal epithelial cells [22]. The crucial roles of claudin-1 as a tumor suppressor or promoter in cancer development and progression have been reported in several studies [23]. The negative correlation of claudin-1 with migration/invasion activities of CCA cells as demonstrated in the present study is also evident in many reports [13, 24, 25, 26]. For instance, overexpression of CD147 markedly increased migratory activities of CCA cells in accordance with decreasing expression of claudin-1 [24]. Suppression of polypeptide N-acetylgalactosaminyltransferase 5 (GLANT5) expression could reduce migration/invasion of CCA cells in conjunction with an increase of claudin-1 protein, and the reverse observation was demonstrated in GLANT5 overexpressing cells [25].

In summary, FOXM1c was shown to be the major isoform found in CCA tissues in association with worse prognosis, advanced stage tumor and short survival of CCA patients. High expression of FOXM1c promoted the migration and invasion activities in CCA cell lines. Expression of claudin-1, a tight junction protein, was negatively correlated with FOXM1 expression. The mechanistic insight of FOXM1c and its

downstream targeted relationship with CCA progression should be further investigated. FOXM1 may be a future therapeutic target of the metastatic CCA.

Declarations

Author contribution statement

Nathakan Klinhom-on: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wunchana Seubwai, Kanlayanee Sawanyawisuth, Sopit Wongkham: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Worachart Lert-itthiporn, Sakda Waraasawapati: Analyzed and interpreted the data.

Marutpong Detarya: Performed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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