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Promoter hypermethylation regulates UCHL1 expression in cholangiocarcinoma cell lines

Phatchareeporn Choodetwattana[1,2], Siriporn Proungvitaya[2,3], Patcharee Jearanaikoon[2,3], Waraporn Chan-on[4], Temduang Limpaiboon[2,3*]

[1] Biomedical Sciences Program, Graduate School, Khon Kaen University, Khon Kaen, Thailand.
[2] Centre for Research and Development of Medical Diagnostic Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[3] Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.
[4] Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Nakhon Pathom, Thailand.

KEYWORDS
Hypermethylation; Silencing; *UCHL1*; 5-azacytidine.

ABSTRACT

Cholangiocarcinoma (CCA) is a significant health burden in Northeastern Thailand. Aberrant DNA methylation is a characteristic feature of most human cancers. Although overexpression of UCHL1 was observed in primary hilar CCA, the regulation of *UCHL1* expression by DNA methylation in CCA has not yet been confirmed. We herein detected DNA methylation and mRNA expression levels in CCA and immortalized cholangiocyte cell lines using MS-HRM and RT-PCR, respectively. The results showed inverse correlation between DNA methylation levels and mRNA expression levels in CCA cell lines. To confirm whether DNA methylation regulated *UCHL1* expression in CCA, 5-azacytidine was applied to inhibit DNA methylation process. We found that UCHL1 protein was up-regulated while DNA methylation was reduced after treatment suggesting the regulation of *UCHL1* expression by DNA methylation. To study the role of *UCHL1*, CCA cell lines were transfected with vector harboring *UCHL1* gene and control vector. Cell proliferation and chemotherapeutic drug sensitivity were investigated. The results showed no difference between these two groups. Our study reveals that overexpression of *UCHL1* alone is not sufficient to neither inhibit tumor cell growth nor enhance chemoresponsiveness. Nevertheless, *UCHL1* expression is epigenetically control indicating its function as a tumor suppressor by which the key features of *UCHL1* and its partners in CCA are worth investigating.

* **Corresponding author:** Temduang Limpaiboon, MT, PhD. Centre for Research and Development of Medical Diagnostic Laboratory, Khon Kaen University, Khon Kaen, Thailand. E-mail: temduang@kku.ac.th
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Introduction

Cholangiocarcinoma (CCA) is a malignant tumor originating from the epithelial cells of bile ducts. The etiology of CCA is usually related to chronic inflammation and biliary duct cell injury induced by the obstruction of bile flow. Previous studies demonstrated that inflammation causes accumulation of aberrant epigenetic alterations[1-3].

DNA methylation is an epigenetic process which influences gene expression without altering the DNA sequence. This process arises from the transfer of methyl groups (CH_3) to the 5' position of cytosine nucleotides of CpG islands by DNA methyltransferases (DNMT). Normal DNA methylation levels are important for the development and function of healthy cells while abnormal DNA methylation levels may contribute to the initiation and progression of most cancers such as retinoblastoma[4], lung cancer[5], breast cancer[6], and CCA[7]. Global DNA hypomethylation may cause genome instability and DNA hypermethylation can lead to silencing of tumor suppressor genes[8,9].

UCHL1 (ubiquitin carboxyterminal hydrolase 1) is a deubiquitinating enzyme that plays role in protein stabilization[10]. The function of *UCHL1* is cleavage of conjugated ubiquitin (Ub) from targeted proteins. The role of *UCHL1* is controversial as there is conflicting evidence regarding its dual oncogenic or tumor suppressor properties depending on cancer type. For oncogenic function, UCHL1 has been shown to be overexpressed in breast cancer cells and promotes invasion[11]. UCHL1 stabilizes cyclin B1 through deubiquitination pathway and promotes cell cycle progression in uterine serous carcinoma[12]. In contrast, *UCHL1* is described as a tumor suppressor in pancreatic neuroendocrine tumors since loss of UCHL1 expression is associated with tumor metastasis. Aberrant DNA methylation of *UCHL1* is associated with prognosis of CCA patients. Nanok et al. (2018) demonstrated that the DNA methylation levels of *UCHL1* were associated with patients' overall survival[13]. CCA patients who had high methylation level of *UCHL1* exhibited significantly shorter survival than those who had low methylation. Similar result was also obtained in 5-fluorouracil-treated CCA patients. They speculated that loss of *UCHL1* expression was due to promoter hypermethylation resulting in the deregulation of *p53* function in apoptosis induction. Nevertheless, the effect of DNA methylation on *UCHL1* expression in CCA has not yet been confirmed. Hence, we aimed to address whether DNA methylation regulated *UCHL1* expression in CCA cell lines. Moreover, the role of UCHL1 in cancer is still unclear, for example, ectopic expression of *UCHL1* promotes proliferation in colorectal cancer cells and tumor volume in xenograft mice[14]. On the other hand, ectopic expression of *UCHL1* significantly reduces the colony formation and proliferation of breast cancer cells via G0/G1 cell cycle arrest and apoptosis induction[15]. Accordingly, in this study, we aimed to elucidate the role of *UCHL1* in CCA cell lines.

Materials and methods

Cell lines and cell culture

Intrahepatic CCA cell lines (KKU-100 and KKU-M055) were established in the Cholangiocarcinoma Research Institute, Khon Kaen, Thailand[16]. Immortalized cholangiocyte cell line (MMNK-1) was established and characterized at Okayama University[17]. Cells were cultured in Dulbecco's Modified Essential Medium (Gibco-BRL, Ontario, Canada) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco-BRL) at 37 °C in a 5% CO_2 atmosphere.

DNA extraction and Bisulfite modification

DNA from cell lines was extracted using Qiagen® Blood & Tissue kit (Qiagen, Hilden, Germany) and quantitated by spectrophotometry at 260 nm. One microgram of DNA was treated with bisulfite using EZ DNA Methylation Gold Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. The modified DNA should be used immediately or stored at -20 °C until it was used as a template for methylation sensitive-high resolution melting (MS-HRM) analysis.

Methylation-sensitive high resolution melting (MS-HRM)

The specific primers of CpG islands related to promoters of *UCHL1* gene were performed as described by Nanok et al. (2018)[13]. PCR amplification and HRM were performed on a Rotor-Gene Q (Qiagen, South San Francisco, CA, USA). The reaction mixture was performed in a final volume of 50 µL containing 50 ng of bisulfite-modified DNA, 1X PCR buffer (67 mM Tris, pH 8.4, 16.6 mM ammonium sulfate and 0.1% Tween 20), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 300 nM of each primer, 1.5 µM SYTO®9 (Invitrogen, Carlsbad, CA, USA), 0.5 unit of Platinum Taq DNA polymerase (Invitrogen). The amplification steps were composed of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min. HRM step consisted of reannealing at 60 °C for 90 sec and slowly warmed by continuous acquisition to 99 °C with rising by 0.5 degree each step. The standard of DNA methylation including 100, 75, 50, 25, 10, and 0% were obtained by mixing bisulfite modified fully methylated (100%) and unmethylated DNA sequences. The HRM data were analyzed using Rotor-Gene Q Series Software 2.1.0 (Qiagen). The value of differential fluorescence of each methylation control against 0% methylation was used to generate a standard curve. The linear equation of each MS-HRM was performed in Microsoft Excel 2010 and used for quantification of methylation levels.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cell lines using SV Total RNA Isolation System (Promega Corporation, Madison, WI, USA). The cDNA was synthesized by reverse transcription of RNA template using ImProm-II™ Reverse Transcription System (Promega) according to the manufacturer's protocols. The specific primer sequences were as follows; *UCHL1*: forward 5'- CTGGGATTTGAGGATGGATC -3'; reverse 5'- GTCATCTACCCGACATTGGC -3'. The 50 µL of PCR reaction consisted of 1X PCR buffer (67 mM Tris, pH 8.4, 16.6 mM ammonium sulfate and 0.1% Tween-20), 0.2 µM of each primer, 200 µM of each dNTP, 50 ng of cDNA, 1.5 µM SYTO®9 (Invitrogen), 2 mM $MgCl_2$ and 0.5 unit of Platinum Taq DNA polymerase (Invitrogen). The cycling stage was performed as the following steps: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 30 sec and extension at 72 °C for 1 min. The PCR reaction was carried out using a Rotor-Gene Q (Qiagen). Relative gene expression was analyzed by the comparative Ct method ($2^{-\Delta\Delta Ct}$)[18].

5-azacytidine treatment

The 5-azacytidine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO and prepared as 10 mM stock solution. CCA cell lines with density of $2x10^5$ were seeded in 6-well plates with cultured medium 2 mL/well. Cells were incubated at 37 °C with 5% CO_2 condition for 24 h, after which 2 mL of fresh cultured medium containing 1 µM of 5-azacytidine or 0.01% DMSO control were replaced for every 24 h until 5 days.

Transfection

CCA cell lines with density of $5x10^5$ cells were seeded in 6-well plates (80% confluence). Then, cells were transfected with 2.5 µg of plasmid harboring *UCHL1* gene, pcDNA3.1+/C-(K)DYK vector (GenScript, Piscataway, NJ, USA) and 2.5 µg of control vector using 3.5 µL of lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. After incubation for 24 h, transfected cells were trypsinized and used for further analysis.

Western blot analysis

CCA cell lines were lysed in 1X RIPA Lysis buffer (Millipore, Darmstadt, Germany) containing protease and phosphatase inhibitor. Total protein concentration was measured using the Bradford assay (Bio-Rad, Hercules, CA, USA). Thirty micrograms of protein samples were separated on a 12.5% SDS-PAGE and transferred onto nitrocellulose membranes. After non-specific block with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T), membranes were incubated overnight at 4°C with primary antibodies against UCHL1 dilution 1: 1,000 (Cell signaling #3524, Beverly, MA, USA) or alpha-tubulin dilution 1: 10,000 (Cell signaling

#2144S). Membranes were washed with TBS-T, incubated at room temperature with goat anti-rabbit IgG dilution 1: 5,000 (Cell signaling #7074) for 1 h, and signal was detected using ECL Western Blotting Detection Reagents (GE Healthcare Life Sciences, Chicago, IL, USA).

Cell proliferation assay

Cell proliferation was measured by CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). Briefly, 5,000 cells were seeded to each well of a 96-well plate. After incubation for 24, 48, and 72 h, MTS solution (Promega) was added to each well and further incubated at 37 °C with 5% CO_2 for 2 h before absorbance is being measured at 490 nm using a Synergy HTX (BioTek, Winooski, VT, USA).

Cytotoxicity assay

Cells with density of 3,000 were seeded in triplicate in a flat-bottom 96-well plate and allowed to grow for 24 h. Then, 100 µL of medium containing different concentrations of gemcitabine were added to each well at the concentration of 0, 10, 20, 40, 80, and 160 nM, respectively. After 72 h, cell viability was performed using MTS assay. Moreover, 100 µL of medium containing different concentrations of cisplatin were added to each well at the concentration of 0, 0.25, 0.5, 1, 2, and 4 µg/mL, respectively. After 48 h, cell viability was performed using MTS assay.

Statistical analysis

The statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The comparison of methylation levels, gene expression, cell proliferation, and cytotoxicity assay were analyzed using Student's t-test. *p*-value < 0.05 was considered statistically significant.

Results

UCHL1 is highly methylated and lowly expressed in CCA cell lines

We used MS-HRM for analyzing the DNA methylation levels of the *UCHL1* promoter in CCA and immortalized cholangiocyte cell lines (MMNK-1). The results showed that DNA methylation levels were significantly high in KKU-100 and KKU-M055 when compared to MMNK-1 (Figure 1A). This is consistent with the relative mRNA expression of *UCHL1* that was abundantly expressed in MMNK-1 cell line, but silenced in CCA cell lines (Figure 1B).

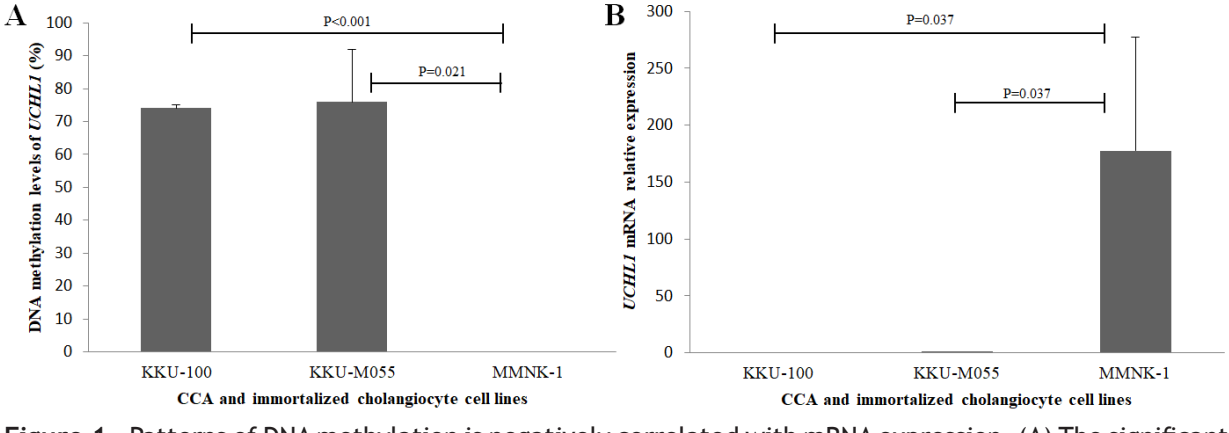

Figure 1 Patterns of DNA methylation is negatively correlated with mRNA expression. (A) The significant differences in DNA methylation between CCA cell lines and MMNK-1. (B) The significant differences in mRNA expression between CCA cell lines and MMNK-1.

Upregulation of UCHL1 in CCA cells following demethylation

To address whether DNA methylation was the cause of *UCHL1* silencing, KKU-100 and KKU-M055 CCA cell lines were treated with the demethylating agent, 5-Azacytidine. As a result of treatment, CCA cell lines showed the significant reduction of DNA methylation levels (Figure 2A) and restoration of UCHL1 protein in KKU-M055 (Figure 2B). These results suggested that DNA hypermethylation in the promoter region of *UCHL1* is responsible for its transcriptional silencing.

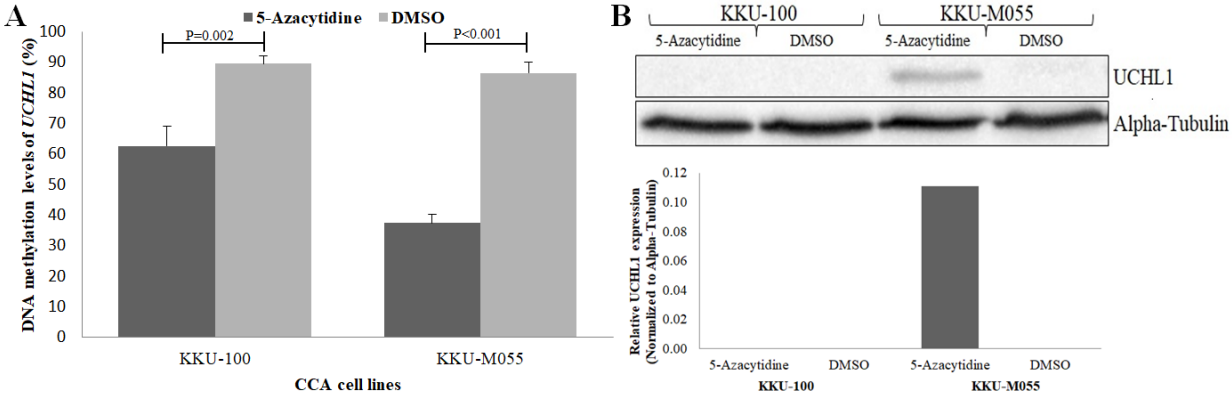

Figure 2 Effects of 5-azacytidine on DNA methylation and protein expression. (A) DNA methylation levels of *UCHL1* in KKU-100 and KKU-M055 were significantly reduced and (B) UCHL1 protein was re-expressed in KKU-M055 after 5-azacytidine treatment.

Re-expression of UCHL1 protein in CCA cell lines and the effect of UCHL1 overexpression on cell proliferation and chemotherapeutic drug sensitivity

Due to the low expression of *UCHL1* in CCA cell lines, we transfected UCHL1 vector into CCA cell lines to restore UCHL1 protein and study the effect of *UCHL1* on cancer phenotype while pcDNA3.1+/C-(K)DYK vector was used as a control. As shown in figure 3A, the transfection was successful as UCHL1 protein was expressed in CCA cell lines. Since the level of UCHL1 protein expression in KKU-100 was low; therefore, we used only KKU-M055 for further studies.

To address whether *UCHL1* played a role in anti-proliferation in CCA, MTS was performed in KKU-M055 transfected cells. The result showed that the overexpression of UCHL1 could not suppress the proliferation of KKU-M055 (Figure 3B). To address whether *UCHL1* played a role in chemotherapeutic drug sensitivity, KKU-M055 transfected cell was treated with various concentrations of gemcitabine for 72 h and cisplatin for 48 h, then MTS assay was performed. The result showed that *UCHL1* had neither effect on gemcitabine (Figure 3C) nor cisplatin (Figure 3D) sensitivity.

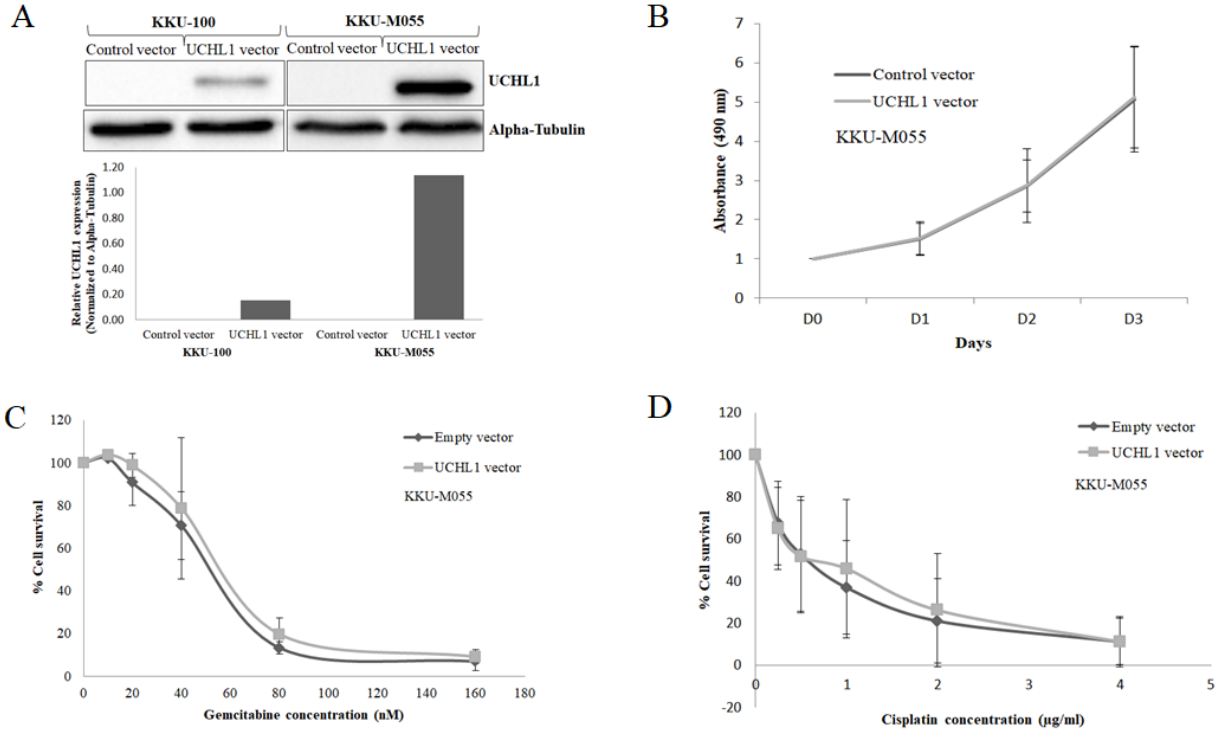

Figure 3 Effect of *UCHL1* overexpression on cell proliferation and chemotherapeutic drug sensitivity. (A) Transfection of the UCHL1 vector into CCA cell lines. The effect of UCHL1 protein overexpression on (B) Cell proliferation, (C) Gemcitabine sensitivity, and (D) Cisplatin sensitivity.

Discussion

Aberrant DNA methylation in cancer has been observed in specific genes. One of them is *UCHL1* which has been reported to be silenced by promoter hypermethylation in many types of cancer such as breast cancer[15], prostate cancer[19], renal cell carcinoma[20], colorectal cancer[21], ovarian cancer[22] and nasopharyngeal carcinoma[23]. These results were consistent with our studies which high methylation of *UCHL1* and low expression of this gene were found in CCA cell lines, indicating that DNA methylation is the major cause for *UCHL1* silencing in CCA cell lines.

Moreover, our study revealed that *UCHL1* was re-expressed by epigenetic drug which was consistent with the study of Xiang et al. (2012)[15]. Although DNA methylation level was significantly decreased in KKU-100 after 5-azacytidine treatment, UCHL1 protein could not be detected. This finding suggested that 5-azacytidine could demethylate *UCHL1* in CCA cell lines but KKU-100 may require either higher dose or longer time treatment than KKU-M055 to restore UCHL1 protein because its doubling time was much longer than that of KKU-M055 (72 h *vs* 25 h)[16,24]. An increase of methylation level observed in KKU-100 and KKU-M055 after treatment with DMSO (Figure 1A VS Figure 2A) was evident by the study of Yokochi and Robertson (2004)[25]. They showed that DMSO stimulates catalytic activity of *de novo* DNA methyltransferase 3a (DNMT3a) *in vitro*.

Functionally, overexpression of *UCHL1* has been found to suppress cell proliferation in breast cancer cells[15] while knockdown of *UCHL1* has been found to reduce the reversal effect of verapamil on chemoresistance to Adriamycin (Doxorubicin trade name) in hepatocellular carcinoma cells[26] and the increase of cisplatin resistance in ovarian cancer cells[22]. Moreover, previous study has shown the association between

UCHL1 protein and TP53 by which UCHL1 protein stabilizes TP53 from proteasome degradation. The study of Li et al. (2010) demonstrated that UCHL1 protein could activate the TP53 signaling pathway by deubiquitinating TP53 and $p14^{ARF}$ protein. The stable $p14^{ARF}$ protein has been the cause of MDM2 inhibition and TP53 accumulation[23]. In our study, no significant alteration of cell proliferation property and chemotherapeutic drug sensitivity were found in *UCHL1* overexpressing CCA cell line. The IARC TP53 Database showed that most of CCA cell lines harbor *p53* mutation. Hence, if the impact of *UCHL1* on cell proliferation and chemosensitivity was influenced by *p53* signaling, it was reasonable for our result that overexpression of UCHL1 in CCA cell line was unable to affect a change in cancer phenotype. Furthermore, some tumor suppressor genes which were stabilized by UCHL1 protein activity may also have promoter hypermethylation resulting in gene silencing. Chinnasri et al. (2008) showed that DNA methylation is the main mechanism of $p14^{ARF}$ inactivation in CCA leading to the loss of $p14^{ARF}$ protein expression[27]. If TP53 was indirectly controlled by UCHL1 via $p14^{ARF}$/*MDM2*/*p53* pathway, it would lead to the results with unchanged cancer phenotype after overexpression of *UCHL1*. Therefore, combined overexpression of *UCHL1* and its targeted genes may lead to significant change in cancer phenotype. To clarify this point, combined overexpression of *UCHL1* and its target, $p14^{ARF}$, in CCA cell lines expressing wild type *p53* will lead to TP53 accumulation contributing to apoptotic induction when treated with chemotherapeutic drug.

Conclusion

We have shown that the expression of *UCHL1* in CCA is regulated by DNA methylation and UCHL1 only by itself is robustly unable to play a crucial role in CCA; it must work in concert with other active molecules to be actionable.

Take home messages

DNA methylation plays a key role in UCHL1 expression.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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References

1. Valinluck V, Sowers LC. Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. Cancer Res 2007; 67(3): 946-50.
2. Abu-Remaileh M, Bender S, Raddatz G, Ansari I, Cohen D, Gutekunst J, et al. Chronic inflammation induces a novel epigenetic program that is conserved in intestinal adenomas and in colorectal cancer. Cancer Res 2015; 75(10): 2120-30.
3. Maiuri AR, Li H, Stein BD, Tennessen JM, O'Hagan HM. Inflammation-induced DNA methylation of DNA polymerase gamma alters the metabolic profile of colon tumors. Cancer Metab 2018; 6: 9.
4. Greger V, Passarge E, Hopping W, Messmer E, Horsthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 1989; 83(2): 155-8.
5. Brock MV, Hooker CM, Ota-Machida E, Han Y, Guo M, Ames S, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med 2008; 358(11): 1118-28.
6. Reyngold M, Turcan S, Giri D, Kannan K, Walsh LA, Viale A, et al. Remodeling of the methylation landscape in breast cancer metastasis. PLoS ONE 2014; 9(8): e103896.
7. Zhang C, Zhang B, Meng D, Ge C. Comprehensive analysis of DNA methylation and gene expression profiles in cholangiocarcinoma. Cancer Cell Int 2019; 19: 352.
8. Tsai H-C, Baylin SB. Cancer epigenetics: linking basic biology to clinical medicine. Cell Res 2011; 21(3): 502-17.

9. Pfeifer GP. Defining driver DNA methylation changes in human cancer. Int J Mol Sci 2018; 19(4): 1166.
10. Bishop P, Rocca D, Henley JM. Ubiquitin C-Terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction. Biochem J 2016; 473(16): 2453-62.
11. Luo Y, He J, Yang C, Orange M, Ren X, Blair N, et al. UCH-L1 promotes invasion of breast cancer cells through activating Akt signaling pathway. J Cell Biochem 2018; 119(1): 691-700.
12. Kwan SY, Au-Yeung CL, Yeung TL, Rynne-Vidal A, Wong KK, Risinger JI, et al. Ubiquitin Carboxyl-Terminal Hydrolase L1 (UCHL1) promotes uterine serous cancer cell proliferation and cell cycle progression. cancers (Basel) 2020; 12(1): 118.
13. Nanok C, Jearanaikoon P, Proungvitaya S, Limpaiboon T. Aberrant methylation of HTATIP2 and UCHL1 as a predictive biomarker for cholangiocarcinoma. Mol Med Rep 2017; 17(3): 4145-53.
14. Zhong J, Zhao M, Ma Y, Luo Q, Liu j, Wang J, et al. UCHL1 acts as a colorectal cancer oncogene via activation of the ß-catenin/TCF pathway through its deubiquitinating activity. Int J Mol Med 2012; 30(2): 430-6.
15. Xiang T, Li L, Yin X, Yuan C, Tan C, Su X, et al. The ubiquitin peptidase UCHL1 induces G0/G1 cell cycle arrest and apoptosis through stabilizing p53 and is frequently silenced in breast cancer. PLoS One 2012; 7(1): e29783.
16. Sripa B, Leungwattanawanit S, Nitta T, Nitta T, Wongkham C, Bhudhisawasdi V, et al. Establishment and characterization of an opisthorchiasis-associated cholangiocarcinoma cell line (KKU-100). World J Gastroenterol 2005; 11(22): 3392-7.
17. Maruyama M, Kobayashi N, Westerman KA, Sakaguchi M, Allain JE, Totsugawa T, et al. Establishment of a highly differentiated immortalized human cholangiocyte cell line with SV40T and hTERT. Transplantation 2004; 77(3): 446-51.
18. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nature protocols 2008; 3: 1101-8.
19. Ummanni R, Jost E, Braig M, Lohmann F, Mundt F, Barett C, et al. Ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) is a potential tumour suppressor in prostate cancer and is frequently silenced by promoter methylation. Mol Cancer 2011; 10(1): 129.
20. Kagara I, Enokida H, Kawakami K, Matsuda R, Toki K, Nishimura H, et al. CpG hypermethylation of the UCHL1 gene promoter is associated with pathogenesis and poor prognosis in renal cell carcinoma. J Urol 2008; 180(1): 343-51.
21. Okochi-Takada E, Nakazawa K, Wakabayashi M, Mori A, Ichimura S, Yasugi T, et al. Silencing of the UCHL1 gene in human colorectal and ovarian cancers. Int J Cancer 2006; 119(6): 1338-44.
22. Jin C, Yu W, Lou X, Zhou F, Han X, Zhao N, et al. UCHL1 is a putative tumor suppressor in ovarian cancer cells and contributes to cisplatin resistance. J Cancer 2013; 4(8): 662-70.
23. Li L, Tao Q, Jin H, Hasselt AV, Poon FF, Wang X, et al. The tumor suppressor UCHL1 forms a complex with p53/MDM2/ARF to promote p53 signaling and is frequently silenced in nasopharyngeal carcinoma. Clin Cancer Res 2010; 16(11): 2949-58.
24. Hematulin A, Meethang S, Utapom K, Wongkham S, Sagan D. Etoposide radiosensitizes p53-defective cholangiocarcinoma cell lines independent of their G2 checkpoint efficacies. Oncol Lett 2018; 15(3): 3895-903.
25. Yokochi T, Robertson KD. Dimethyl sulfoxide stimulates the catalytic activity of de novo DNA methyltransferase 3a (Dnmt3a) in vitro. Bioorg Chem 2004; 32(4): 234-43.
26. Yang G, Fan G, Zhang T, Ma K, Huang J, Liu M, et al. Upregulation of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) mediates the reversal effect of verapamil on chemoresistance to adriamycin of hepatocellular carcinoma. Med Sci Monit 2018; 24: 2072-82.
27. Chinnasri P, Pairojkul C, Jearanaikoon P, Sripa B, Bhudhisawasdi V, Tantimavanich S, et al. Preferentially different mechanisms of inactivation of 9p21 gene cluster in liver fluke–related cholangiocarcinoma. Hum Pathol 2009; 40(6): 817-26.