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Ras-ERK1/2 signaling accelerates the progression of colorectal cancer via mediation of H2BK5ac

Huanxiang Jia[a,1], Ming Xu[b,1], Yan Bo[c], Wenxiao Li[d], Runhe Zhou[c,*]

[a] Department of Radiology, Jining No.1 People's Hospital, Jining 272011, Shandong, China
[b] Department of Anorectal Surgery, Qilu Hospital of Shandong University (Qingdao), Qingdao 266035, Shandong, China
[c] Department of General Surgery, Qilu Hospital of Shandong University (Qingdao), Qingdao 266035, Shandong, China
[d] Department of Intensive Care Unit, Shandong Provincial Third Hospital, Jinan 250000, Shandong, China

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ABSTRACT

Aims: Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) is a key downstream gene of Ras pathway. Activation of Ras-ERK1/2 has been testified to be linked to the progression of diverse cancers. Nonetheless, whether Ras-ERK1/2-tumorigenic pathway is mediated by epigenetic factors remains indistinct. The purpose of the research attempted to disclose the functions of H2BK5ac in Ras-ERK1/2-evoked CRC cell phenotypes.

Materials and methods: Western blot assay was implemented for exploration of the relevancy between Ras-ERK1/2 and H2BK5ac. H2BK5Q was established and its functions in cell viability, colony formation and migration were appraised via utilizing MTT, soft-agar colony formation and Transwell assays. The mRNA and transcription of ERK1/2 downstream genes were estimated via RT-qPCR and ChIP assays. HDAC2 functions in SW48 cell phenotypes were evaluated after co-transfection with pEGFP-Ras$^{Q61L/T35S}$ and si-HDAC2 vectors. Additionally, the involvements of ATF2 and MDM2 in Ras-ERK1/2-affected H2BK5ac expression were estimated.

Key findings: H2BK5ac expression was evidently repressed by Ras-ERK1/2 pathway in SW48 cells. Moreover, Ras-ERK1/2-elevated cell viability, the number of colonies and migration were both impeded by H2BK5ac. The mRNA and transcriptions of CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16 were both mediated by H2BK5ac. Additionally, HDAC2 silence overtly recovered H2BK5ac expression inhibited by Ras-ERK1/2, meanwhile abated Ras-ERK1/2-affected SW48 cell phenotypes. Beyond that, restrained H2BK5ac induced by Ras-ERK1/2 was concerned with MDM2-mediated ATF2 degradation.

Significance: These investigations testified that Ras-ERK1/2 pathway affected SW48 cell phenotypes through repressing H2BK5ac expression. Otherwise, declined H2BK5ac might be linked to MDM2-mediated ATF2 degradation.

1. Introduction

Colorectal cancer (CRC) is also called as colon cancer or bowel cancer, which is a pervasive malignant tumor of the digestive tract developing from the colon or rectum [1]. In 2017, CRC statistic report manifested that the estimated new CRC cases reached 135,430 individuals, and the deaths cases reached 50,260 individuals in United States [2]. The occurrence of CRC is basically with a high-fat and low-cellulose diet, and is also associated with age and lifestyle factors [3,4]. In accordance with the physical condition of CRC patients and the stage of the tumor, the complete surgical removal is the preferred therapeutic strategies for CRC [5]. The diagnosis and remedy of CRC require the joint effort of clinician and nurse personnel. More importantly, scientific and effective nursing intervention can reduce the occurrence of complications of CRC [6]. Nonetheless, when CRC is caught at later stage accompanied by metastases, clinician can only relieve the tumor symptoms of CRC patients via palliative treatment [7]. Hence, further exploration of the neoteric and valid remedial ways for CRC is still imperative.

Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) is a momentous signaling pathway, and its diversiform functions have been corroborated in numerous cancers comprising CRC [8,9]. ERK1/2 is a pivotal downstream signaling molecule of Ras pathway, which can be activated by Ras pathway persistently [10]. Activation of Ras-ERK1/2

* Corresponding author at: Department of General Surgery, Qilu Hospital of Shandong University (Qingdao), No.758, Hefei Road, Qingdao 266035, Shandong, China.
E-mail address: zhou14runhe@sina.com (R. Zhou).
[1] Equal first authors.

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pathway has been testified to impair neuronal cell migration, neurogenesis and synapse formation [11]. Moreover, Ras-ERK1/2 pathway has corroborated to be involved in adjusting cell proliferation and invasion processes in gastric cancer and in mediating cell apoptosis in skin tumorigenesis [12,13]. A crucial research illustrated that activation of ERK1/2 might be a molecular biomarker for CRC [14]. Park et al. disclosed that Esculetin could hinder CRC cell proliferation via mediating Ras-ERK1/2 pathway [15]. On the basis of these anterior researches, the depth regulatory mechanisms of Ras-ERK1/2 pathway in CRC have drawn our extensive research interest.

Present studies attested that abnormality of epigenetic information, especially histone modification, is intimately correlated with the evolution of human malignant tumors [16,17]. Emerging evidence displayed that Ras-ERK1/2 pathway could affect the development of CRC via mediating H3K9ac [18]. Histone acetylation at H2B lysine 5 (H2BK5ac) is a vital form of histone H2B, which has been testified to be linked to gene transcription and to participate in controlling epithelial to mesenchymal transition (EMT) process [19,20]. Nonetheless, whether H2BK5ac can affect Ras-ERK1/2 pathway-regulated CRC cell phenotypes remains vague. In the actual research, we probed the relevancy between Ras-ERK1/2 pathway and H2BK5ac for uncovering the latent regulatory mechanism of Ras-ERK1/2 on the development of CRC.

2. Materials and methods

2.1. Cell culture and disposition

CRC cell line of SW48 was acquired from American Type Culture Collection (ATCC® CCL-231™, Rockville, MD, USA). SW48 cells were fostered in ATCC-recommend Leibovitz's L-15 medium (ATCC® 30-2008™), which incorporated 10% FBS (Hyclone, Logan, UT, USA), meanwhile were cultivated at 37 °C comprising 100% air. MG132 (≥95%, an inhibitor of proteasome) was bought from Sigma (St. Louis, MO, USA) and were utilized to dispose SW48 cells for 0, 3, 6 and 12 h.

2.2. Plasmid construction and cell transfection

The coding regions of H2B and the wild-type N-Ras were amplified by exploiting PCR and were sub-cloned into p-EGFP-N1 (Clontech, Basingstoke, UK). pEGFP-N-Ras$^{Q61L/T35S}$ vector was attained by utilizing site-directed mutagenesis. The pEGFP-H2BK5Q was established via adopting the TaKaRa MutanBEST Kit (TaKaRa, Dalian, China). The diverse siRNA sequences that specific for HDAC2 (si-HDAC2-1 and si-HDAC2-2) and MDM2 (si-MDM2) were synthesized from GenePharma (Shanghai, China). The amplified activating transcription factor-2 (ATF2) and HDAC2 were sub-cloned into HA-tag vector (Invitrogen, Carlsbad, CA, USA). MDM2-His and MDM2-MU were bought from Abace Biotechnology Co., Ltd. (Beijing, China). For cell transfection, the above-mentioned vectors were transfected into SW48 cells via utilizing Lipofectamine 3000 (Invitrogen). After transfection for 48 h, SW48 cells were gathered and employed for the following experiments.

2.3. Determination of cell viability

After transfection, the viability of SW48 cells was estimated through employing MTT assay (Sigma). The transfected SW48 cells were fostered in 96-well plate, and subsequently 20 μL MTT solution was mixed into the culture plate. After co-cultivating for supererogatory 1 h and shocking for 10 s, the absorbance at 450 nm was appraised via adopting a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.4. Soft-agar colony formation assay

SW48 cells were transfected with pEGFP-N1, pEGFP-H2B, pEGFP-Ras$^{Q61L/T35S}$ and H2BK5Q. After 48 h transfection, cells were gathered and suspended in DMEM medium incorporating 0.35% low-melting agarose. Afterward, cells were replenished to the solidified 0.6% agar and co-cultivated for 3–4 weeks at 37 °C. After incubation, the number of colonies was counted via utilizing a microscope (Olympus, Tokyo, Japan).

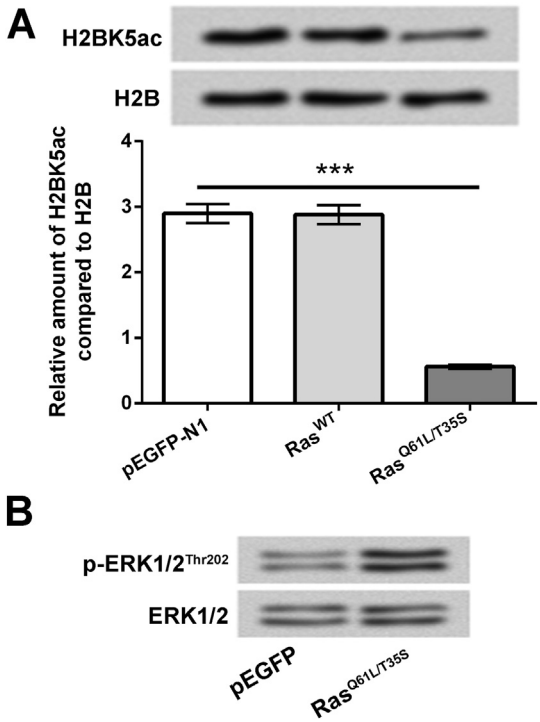

Fig. 1. H2BK5ac expression was repressed by Ras-ERK1/2 pathway.
(A) Disparate vectors of pEGFP-N1, pEGFP-Ras^{WT} and pEGFP-Ras$^{Q61L/T35S}$ were transfected into SW48 cells, and H2BK5ac protein level was evaluated via western blot assay. (B) p-ERK1/2^{Thr202} and ERK1/2 protein level in pEGFP-N1 and pEGFP-Ras$^{Q61L/T35S}$-transfected SW48 cells were appraised through executing western blot assay. ***$P < 0.001$.

2.5. Detection of cell migration

For cell migration assessment, Transwell assay with 8-μm pore filters (Costar, Boston, MA, USA) was executed. After transfection, SW48 cells were fostered in the non-serum culture medium and replenished to the upper chamber of Transwell equipment. The lower chamber of Transwell was replenished with 600 μL complete culture medium. After fostering for 12 h at 37 °C, the migrated cells in the lower chamber were immobilized with 4% methanol, synchronously stained with 0.5% crystal violet (Sigma) for 20 min. After staining, the absorbance at 570 nm was analyzed via executing Microplate Reader (Bio-Rad).

2.6. Detection of cell cycle

Cell Cycle and Apoptosis Analysis Kit (Beyotime, Shanghai, China) was adopted for cell cycle assessment. After 48 h transfection, SW48 cells were rinsed twice with ice-cold PBS (Beyotime), in the meantime immobilized with 70% ethanol for 2 h at 4 °C. Afterward, the transfected SW48 cells were stained with 25 μg/mL propidium iodide (PI) solution incorporating 0.1% Triton X-100 and 10 μg/mL RNase A (Invitrogen) for 30 min at circumambient temperature. FACScan flow cytometer (Becton Dickinson, San Jose, USA) was executed for assessment of the percentages of cells in G0/G1, S and G2/M phases of cell cycle.

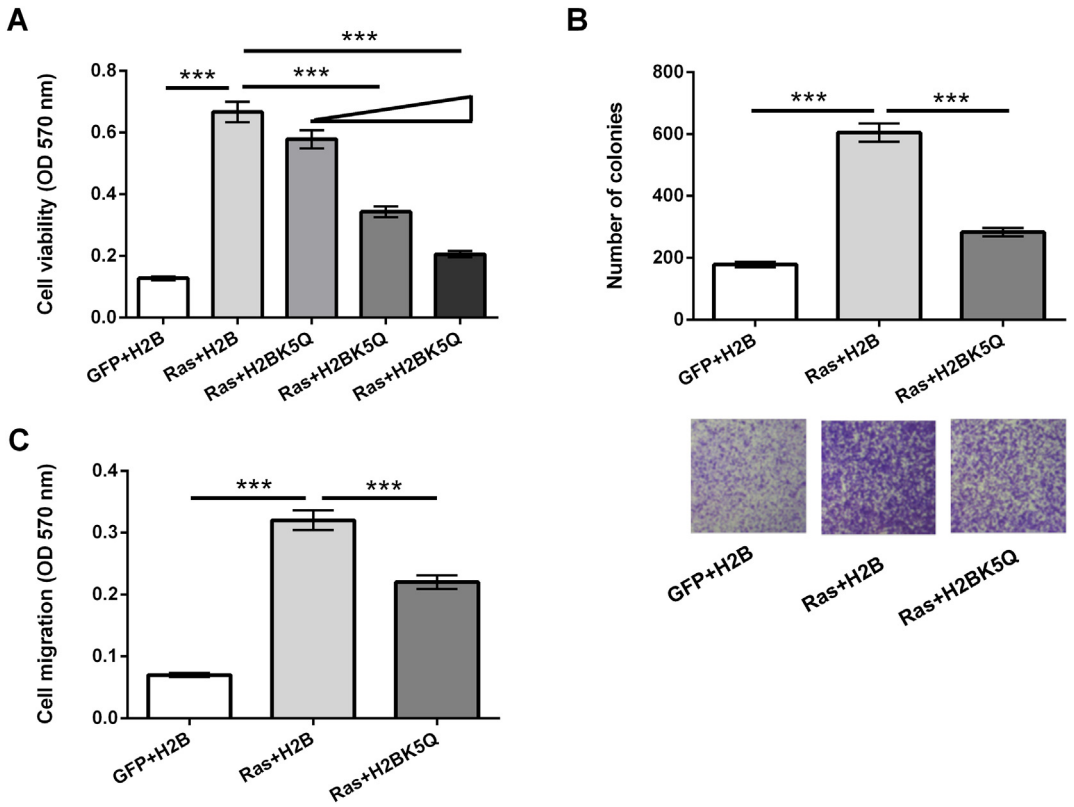

Fig. 2. H2BK5ac restrained Ras-ERK1/2-evoked CRC cell phenotypes.
H2BK5Q (0.5, 1 and 2 μg) was established to imitate the acetylation state of H2B in K5 site. After transfection with pEGFP-N1, pEGFP-H2B, pEGFP-Ras$^{Q61L/T35S}$ and H2BK5Q, (A) the viability of SW48 cells was estimated via preforming MTT assay. The follow-up experiment utilized 2 μg H2BK5Q for SW48 cells transfection. After transfection, (B) the colony numbers and (C) the ability of SW48 cell migration were appraised via executing soft-agar colony formation and Transwell assays. ***$P < 0.001$.

2.7. Reverse transcription-quantitative PCR (RT-qPCR)

Employing TRIzol reagent (Invitrogen) extracted total RNA from the transfected SW48 cells. PrimeScript™ 1st Strand cDNA Synthesis kit (TaKaRa) was executed for reverse transcription. QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany) was utilized for RT-qPCR analysis accompanied by the Mx4000™ quantitative PCR system (Stratagene, La Jolla, CA, USA). GAPDH was regarded as an internal control, meanwhile the classic $2^{-\Delta\Delta Ct}$ method [21] was adopted for numeration of these data.

2.8. Chromatin immunoprecipitation (ChIP)

The transfected SW48 cells were gathered and were cross-linked in 1% formaldehyde (Sigma) for 10 min. For termination of the cross-linking, 1 M glycine (Sigma) was replenished to SW48 cells and reacted for 5 min at circumambient temperature. SW48 cells were subsequently rinsed with PBS and were lysed in SDS Lysis Buffer (Beyotime). The cell lysates were proceeded the ultrasonic treatment via exploiting Bioruptor (Diagenode, Liege, Belgium). After centrifugation for 10 min at 10,000 × *g* at 4 °C, immunoprecipitation was executed via employing anti-H2BK5ac antibody (ab40886, Abcam, Cambridge, MA, USA), anti-ATF2 antibody (#35031, Cell Signaling Technology, Beverly, MA, USA). The anti-IgG antibody (ab2410, Abcam) was utilized as a black control. Subsequently, 60 μL Protein A + G Agarose/Salmon Sperm DNA (Beyotime) was replenished and was co-cultivated for additional 2 h at 4 °C. The beads were rinsed on the basis of the anteriorly described [22]. RT-qPCR assay was executed for analyzing the input DNA.

2.9. Western blot assay

The transfected SW48 cells with diverse vectors were gathered and rinsed twice with PBS. RIPA lysis buffer (Beyotime) with protease inhibitor was utilized for total protein extraction. The frequently-exploited BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA) was executed for determining the protein concentrations. The protein specimens were absolutely separated by utilizing SDS-PAGE, meanwhile were transferred to the nitrocellulose membranes (Bio-Rad). After sealing with BSA-TBST for 1 h, the nitrocellulose membranes were co-cultivated with the extraordinary primary antibodies (1:1000) overnight at 4 °C. The extraordinary primary antibodies of anti-H2BK5ac (ab175046), anti-H2B (ab52599), anti-ERK1/2 (ab184699), anti-HA (ab137838), anti-GFP (ab183734), anti-His (ab9108), anti-ATF2 (ab47476), anti-MDM2 (ab226939), anti-β-actin (ab8227) and anti-p-ERK1/2^{Thr202} (MA5-15173, Invitrogen) were utilized in the correlative experiments. After this, the relevant secondary antibodies were co-cultivated nitrocellulose membranes for supererogatory 2 h at indoor temperature. Enhanced chemiluminescence (ECL) reagent was exploited to estimate the bands, and the intensities of the bands were quantified through employing the Image Lab™ Software (Bio-Rad).

2.10. Statistical analysis

The results in the actual research were emerged as mean ± SD. SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was exploited for statistical analysis. ANOVA along whit Duncan post-hoc was utilized for analyzing *P*-value. The $P < 0.05$ was provided with significant difference.

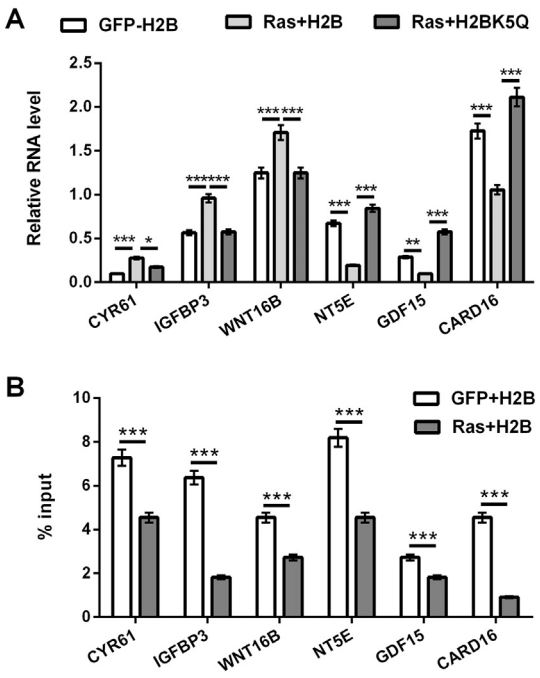

Fig. 3. H2BK5ac mediated the transcription of ERK1/2 pathway downstream target genes.
After pEGFP-N1, pEGFP-H2B, pEGFP-Ras$^{Q61L/T35S}$ and H2BK5Q transfection, (A) CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16 mRNA expression levels were evaluated via implementing RT-qPCR assay; (B) the transcription of above-involved genes was estimated by executing ChIP assay. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3. Results

3.1. H2BK5ac was repressed by Ras-ERK1/2 pathway

We initially established the vectors of pEGFP-Ras$^{Q61L/T35S}$ and pEGFP-Ras^{WT} for exploration of the relevancy between H2BK5ac and Ras-ERK1/2 pathway. Meanwhile, pEGFP-N1 vector was constructed and utilized as a control. In Fig. 1A, we discovered the impeded protein level of H2BK5ac in pEGFP-Ras$^{Q61L/T35S}$-transfected SW48 cells as contrasted to that in pEGFP-N1-transfected cells ($P < 0.001$). The discovery hinted that H2BK5ac might be specifically mediated via the Ras-ERK1/2 pathway. Subsequently, we further verified whether the specific mutant vector (Ras$^{Q61L/T35S}$) can specifically activate the ERK1/2 pathway. Results in Fig. 1B disclosed that p-ERK1/2^{Thr202} protein level was overtly elevated in Ras$^{Q61L/T35S}$ vector-transfected SW48 cells compared with that in pEGFP-N1 vector-transfected cells. This data implied that the specific mutant vector Ras$^{Q61L/T35S}$ indeed activated ERK1/2 pathway specifically.

3.2. H2BK5ac participated in mediating Ras-ERK1/2-evoked CRC cell phenotypes

We next constructed H2BK5Q to imitate the acetylation state of this site, simultaneously co-transfected with Ras$^{Q61L/T35S}$ for probing the influence of H2BK5ac in Ras-ERK1/2-evoked colon cancer cell proliferation and migration. We observed the enhancement of SW48 cell viability after co-transfection with Ras$^{Q61L/T35S}$ and H2B as relative to co-transfection with GFP and H2B ($P < 0.001$, Fig. 2A). But, the accelerative impact was distinctly ameliorated by H2BK5Q in an amount-dependent manner (0.5, 1 and 2 μg, $P < 0.001$, Fig. 2A). Moreover, the colony numbers were also enhanced in SW48 cells after Ras$^{Q61L/T35S}$ and H2B co-transfection, whereas repressed after Ras$^{Q61L/T35S}$ and H2BK5Q co-transfection ($P < 0.001$, Fig. 2B). Outside of this, cell migration was similarly aggrandized in Ras$^{Q61L/T35S}$ and H2B co-transfected SW48 cells and impeded in Ras$^{Q61L/T35S}$ and H2BK5Q co-transfected cells ($P < 0.001$, Fig. 2C). Above observations corroborated that H2BK5ac eliminated the facilitative impacts of Ras-ERK1/2 on SW48 cells proliferation and migration.

3.3. H2BK5ac participated in regulating the downstream target gene transcription of ERK1/2 pathway

To further uncover the influences of H2BK5ac in Ras-ERK1/2 pathway, the impacts of H2BK5ac on the downstream target gene transcription of ERK1/2 pathway was studied. We observed that CYR61, IGFBP3 and WNT16B mRNA expression levels were all elevated, but NT5E, GDF15 and CARD16 mRNA expression levels were all repressed in SW48 cells with Ras$^{Q61L/T35S}$ and H2B co-transfection ($P < 0.01$ or $P < 0.001$, Fig. 3A). In Ras$^{Q61L/T35S}$ and H2BK5Q co-transfected cells, the mediatory impacts of Ras-ERK1/2 pathway on these downstream target genes were apparently overturned ($P < 0.05$ or $P < 0.001$, Fig. 3A). Additionally, we discovered that the enrichment of H2BK5ac was memorably restrained at the promoter regions of CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16 after activation of Ras-ERK1/2 ($P < 0.001$, Fig. 3B). The above-involved data hinted that H2BK5ac could participate in mediating the transcription of ERK1/2 downstream genes.

3.4. Repressed HDAC2 mediated Ras-ERK1/2-evoked CRC cell phenotypes

HDAC2 is a momentous histone deacetylase, which has been testified to be involved in mediating H2B functions. Herein, we probed whether HDAC2 could mediate the impacts of H2BK5ac on colon cancer cell phenotypes. In Fig. 4A, we found that HDAC2 mRNA expression was prominently declined by si-HDAC2-1 and si-HDAC2-2 transfection. Interestingly, we discovered that repression of H2BK5ac evoked by Ras-ERK1/2 was obviously recovered in SW48 cells after si-HDAC2-1 and si-HDAC2-2 transfection. Noticeably, HDAC2 inhibition hindered Ras-ERK1/2-evoked cell viability and migration in SW48 cells ($P < 0.001$, Fig. 4B and C). Moreover, HDAC2 inhibition impeded the percentage of SW48 cells in S phase (Fig. 4D). Beyond that, HDAC2 inhibition repressed CYR61, IGFBP3 and WNT16B mRNA expression, however ascended NT5E, GDF15 and CARD16 mRNA expression following the activation of Ras-ERK1/2 ($P < 0.001$, Fig. 4E). These explorations indicated that repression of HDAC2 recovered Ras-ERK1/2-inhibited H2BK5ac expression and hindered the carcinogenic activities of Ras-ERK1/2 in SW48 cells.

3.5. Suppressed H2BK5ac evoked by Ras-ERK1/2 pathway was associated with the degradation of ATF2

We subsequently probed whether the decrease of H2BK5ac was associated with the mediation of ATF2. The results from exogenous and endogenous experiments disclosed that ATF2 protein level was impeded in SW48 cells after activation of ERK1/2 pathway. But, there was no appreciable alteration of ATF2 mRNA level (Fig. 5A–C). HDAT2 has no change at transcription or protein levels (Fig. 5A–C). Additionally, we observed that ATF2 and H2BK5ac protein levels were both declined by Ras-ERK1/2 activation in SW48 cells (Fig. 5D). Moreover, the enrichment of ATF2 was impeded at the promoters of CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16 genes ($P < 0.001$, Fig. 5E). After administration with MG132 (a proteasome inhibitor), we discovered that Ras-ERK1/2-evoked ATF2 repression was recovered (Fig. 5F). To further verify the consequence, the time gradient experiment was carried out. We observed that H2BK5ac protein level was evidently cut down by Ras$^{Q61L/T35S}$ after 48 h transfection accompanied by MG132 absence (Fig. 5G). Nevertheless, after disposition with 25 μM MG132, H2BK5ac expression was notably recovered in Ras$^{Q61L/T35S}$-transfected cells (Fig. 5H). All above-involved discoveries hinted that Ras-ERK1/2

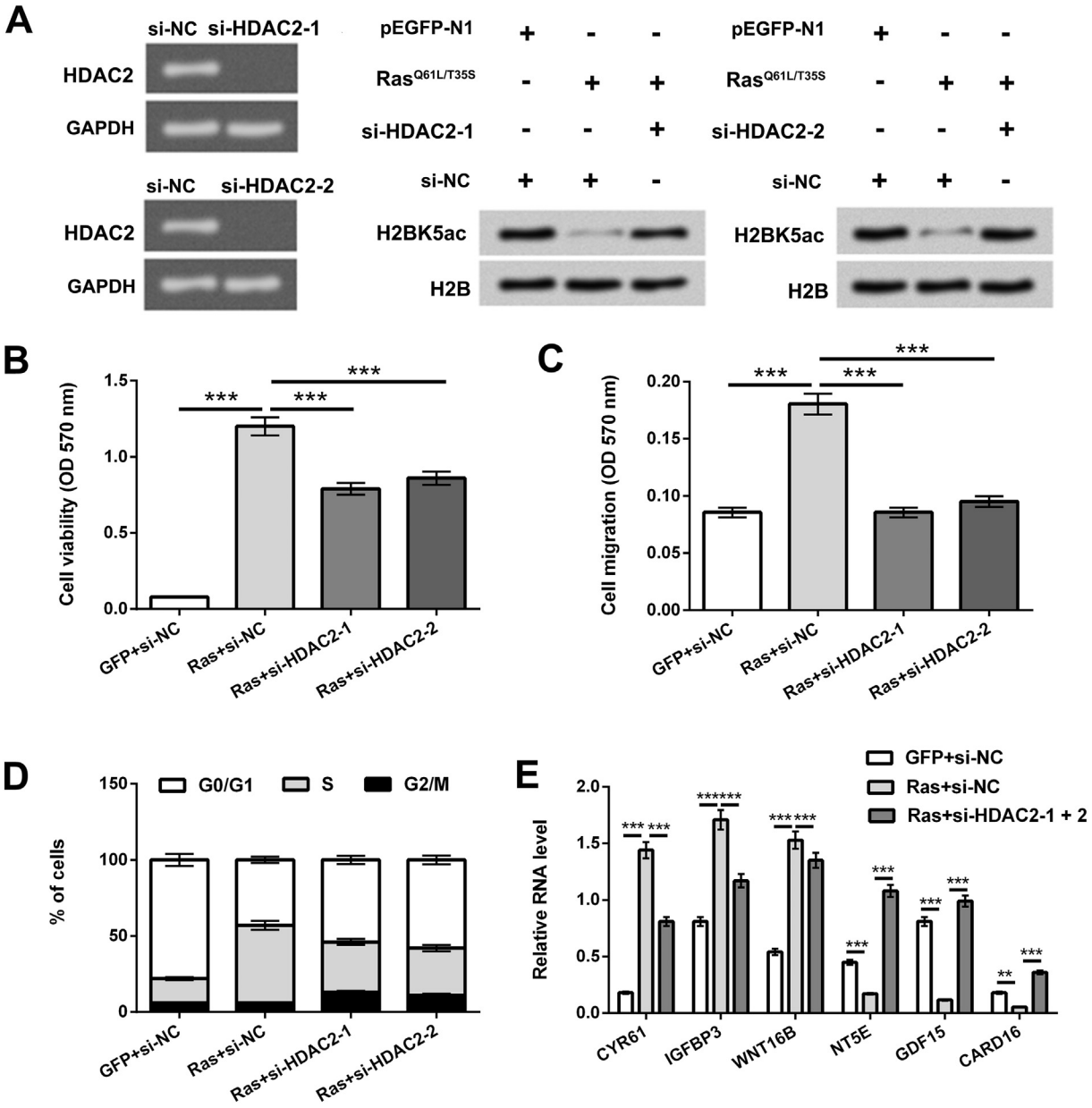

Fig. 4. Repression of HDAC2 affected Ras-ERK1/2-evoked CRC cell phenotypes.
(A) HDAC2 mRNA expression in SW48 cells with si-HDAC2-1 and si-HDAC2-2 transfection was evaluated via carrying out RT-qPCR assay and observed through utilizing gel electrophoresis. Above cells were co-transfected with pEGFP-N1, and pEGFP-Ras$^{Q61L/T35S}$, H2BK5ac protein level was appraised via employing western blot assay. (B) Cell viability, (C) migration, (D) cell cycle process and (E) ERK1/2 downstream genes (CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16) were respectively estimated through executing MTT, Transwell, flow cytometry and RT-qPCR assays. $^{**}P < 0.01$, $^{***}P < 0.001$.

restrained H2BK5ac expression through degradation of ATF2 in SW48 cells.

3.6. Ras-ERK1/2 degraded ATF2 by regulation of MDM2

We ultimately appraise whether MDM2 is involved in the process of ATF2 degradation after activating ERK pathway. Fig. 6A and B displayed that MDM2-His transfection notably elevated MDM2 protein level, but repressed ATF2 protein level in Ras$^{Q61L/T35S}$-transfected SW48 cells (Fig. 6C). After transfection with MDM2-MU, we discovered that the protein level of ATF2 was not declined in Ras$^{Q61L/T35S}$-transfected SW48 cells (Fig. 6D). These consequences implied that the degradation of ATF2 required the involvement of MDM2. Outside of this, we observed that Ras$^{Q61L/T35S}$ elevated MDM2 expression, synchronously declined H2BK5ac expression (Fig. 6E). Next, si-MDM2 and si-NC vectors were transfected into SW48 cells, the repressed expression of MDM2 was observed in si-MDM2-transfected cells (Fig. 6F). Interestingly, the hindered impacts of Ras-ERK1/2 on H2BK5ac expression was evidently eliminated by MDM2 silence (Fig. 6G). All above observations implied that suppressed H2BK5ac evoked by Ras-ERK1/2 pathway was likely to be associated with MDM2-mediated ATF2 degradation.

4. Discussion

Ras gene is one of the earliest identified human oncogenes, which extensively participates in adjusting diverse cell life activities [23]. These regulatory mechanisms are inseparable with its downstream signaling pathways. ERK1/2 is a crucial downstream pathway of Ras, which can be activated through its site mutation [24]. Moreover, Ras

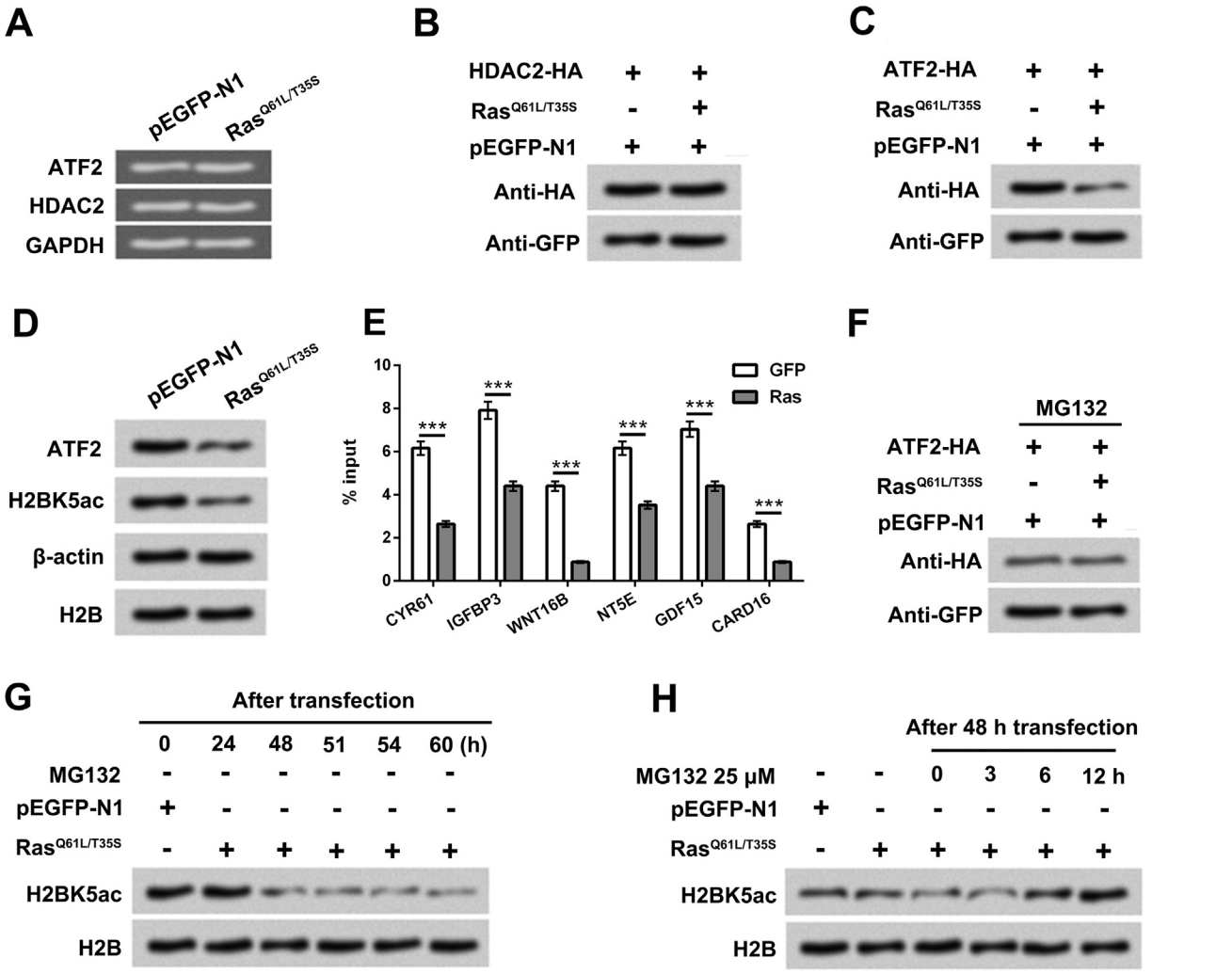

Fig. 5. Ras-ERK1/2 repressed H2BK5ac expression by degrading ATF2.
(A) After pEGFP-N1 and pEGFP-Ras$^{Q61L/T35S}$ transfection, ATF2 and HDAC2 mRNA levels were appraised via executing RT-qPCR assay and observed via gel electrophoresis. (B) After HDAC2-HA and ATF2-HA co-transfection, HDAC2 and ATF2 protein levels were estimated via implementing western blot assay. After pEGFP-N1 and pEGFP-Ras$^{Q61L/T35S}$ transfection, (D) ATF2 and H2BK5ac protein levels and (E) ERK1/2 downstream genes (CYR61, IGFBP3, WNT16B, NT5E, GDF15 and CARD16) were respectively appraised via executing western blot and ChIP assays. (F) After pEGFP-N1, pEGFP-Ras$^{Q61L/T35S}$ and ATF2-HA transfection as well as MG132 (a protease inhibitor) disposition, ATF2 protein level was evaluated via carrying out western blot assay. (G) After transfection with pEGFP-Ras$^{Q61L/T35S}$ for 24, 48, 51 and 60 h, H2BK5ac protein level was evaluated via employing western blot assay. (H) After transfection with pEGFP-Ras$^{Q61L/T35S}$ for 48 h and disposition with MG132 (25 μM) for 3–12 h, H2BK5ac protein level was appraised via performing western blot assay. ***$P < 0.001$.

gene mutation has been certified to be correlated with elevated proliferation and spontaneous apoptosis in CRC cells [25]. In the current research, we discovered that Ras mutated at Q61L and T35S sites indeed activated ERK1/2 pathway in SW48 cells. We subsequently elementarily explored the correlation between Ras-ERK1/2 pathway and H2BK5ac, meanwhile disclosed the influences of H2BK5ac in Ras-ERK1/2-affected CRC cell phenotypes. The discoveries revealed that Ras-ERK1/2 repressed H2BK5ac via MDM2-mediated ATF2 degradation. Outside of this, suppression of H2BK5ac evoked by Ras-ERK1/2 activation conduced to SW48 cell proliferation, migration and the transcription of ERK1/2 downstream genes.

It is exposed that histone modification is implicated in the mediation of the progress of cancers facilitated by Ras signaling [26]. Ras-PI3K-AKT pathway has been discovered to affect histone H3 acetylation at lysine 56 (H3K56ac) expression, thereby adjusting HeLa cells proliferation and migration [27]. Tian et al. corroborated that Ras-ERK1/2 pathway facilitated the progression of CRC through mediation of H3K9ac [18]. Acetylation of histone H2B is also a vital pattern of histone modification, however, there is a little research concentrated on the impacts of acetylation of histone H2B on the pathogenesis of cancers. On the basis of the studies of forerunners, we probe the involvements of H2BK5ac in the carcinogenesis of Ras-ERK1/2 pathway in CRC cells. Our outcomes disclosed that H2BK5ac expression was restrained by Ras-ERK1/2 pathway. Subsequently, H2BK5Q was established to further uncover the influences of H2BK5ac in CRC SW48 cell phenotypes. We observed that H2BK5Q impeded SW48 cell proliferation and migration, hinting that acetylation of histone H2BK5 could affect the progression of CRC accelerated by Ras-ERK1/2 pathway.

ERK1/2 pathway is a pivotal downstream pathway of Ras, which is mediated by multifarious molecules [28]. CYR61, IGFBP3 and GDF15 genes have been recognized as neoteric diagnostic and predictive biomarkers for CRC [29–31]. Moreover, CYR61 and GDF15 genes are involved in adjusting CRC cell EMT and metastasis processes [32,33]. WNT16B, NT5E and CARD16 are momentous downstream genes of ERK1/2 pathway, which have testified to be oncogenes or tumor-suppressive genes in diverse cancers [34–36]. In the recent research, we studied the impacts of H2BK5ac on these above-involved genes, which associated with ERK1/2 pathway. The elevation of CYR61, IGFBP3 and

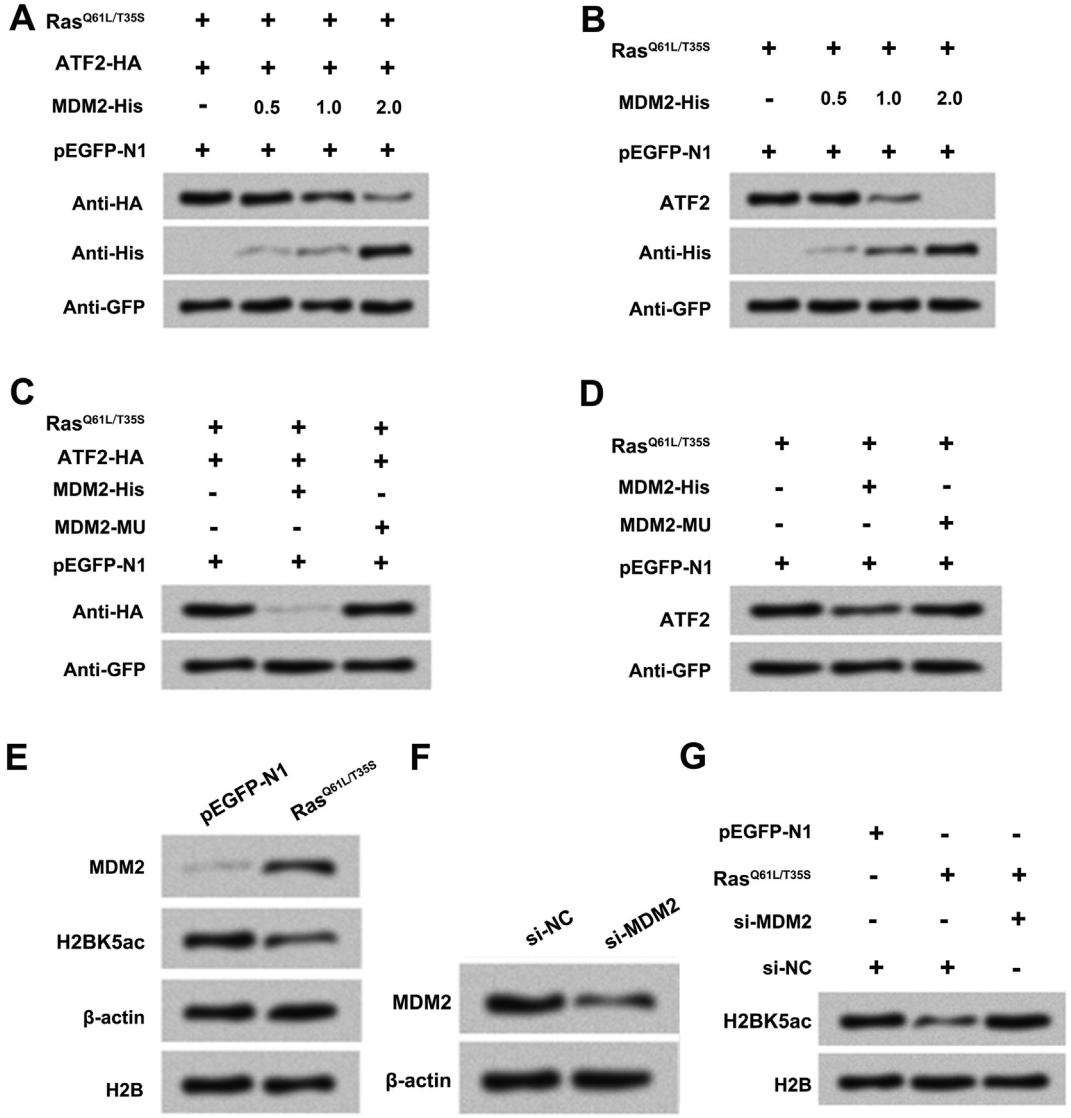

Fig. 6. Ras-ERK1/2 pathway degraded ATF2 by mediation of MDM2.
(A and B) After pEGFP-N1, pEGFP-Ras$^{Q61L/T35S}$, MDM2-His (0.5, 1.0, and 2.0 μg) and ATF2-HA transfection, ATF2 exogenous and endogenous expression levels were estimated via implementing western blot assay. (C and D) After pEGFP-N1, pEGFP-Ras$^{Q61L/T35S}$, MDM2-His, MDM2-MU and ATF2-HA transfection, ATF2 exogenous and endogenous expression levels were evaluated via performing western blot assay. (E) After pEGFP-N1 and pEGFP-Ras$^{Q61L/T35S}$ transfection, MDM2 and H2BK5ac protein levels were appraised via utilizing western blot assay. (F) After si-MDM2 and si-NC transfection, MDM2 protein level was estimated via carrying out western blot assay. (G) After pEGFP-N1, pEGFP-Ras$^{Q61L/T35S}$, si-MDM2 and si-NC transfection, H2BK5ac protein level was evaluated via performing western blot assay.

WNT16B, meanwhile the repression of NT5E, GDF15 and CARD16 were presented in H2BK5ac-transfected SW48 cells. More importantly, the enrichment of H2BK5ac in all above-mentioned gene promoter regions was declined. These findings implied that H2BK5ac could affect the transcription of ERK1/2 pathway downstream genes.

HDAC2 is a key enzyme that catalyzes the deacetylation of histones, and enhancement of HDAC2 has been discovered in CRC patients at the early stage [37]. Moreover, an important research illuminated that repression of HDAC2 could restrain cell viability in CRC HCT116 cells [38]. ATF2 belongs to the leucine zipper transcription factor family, which possesses carcinogenic and anticancer dual functions [39]. An interesting study from Chen et al. uncovered that suppression of histone demethylase JMJD1C could hinder CRC cell metastasis via targeting ATF2 [40]. In accordance with these researches, we probed the functions of HDAC2 and ATF2 in H2BK5ac expression and in CRC cell phenotypes. We discovered that repression of HDAC2 recovered H2BK5ac expression under activation of Ras-ERK1/2 pathway. Simultaneously, repression of HDAC2 impeded Ras-ERK1/2-evoked SW48 cell proliferation and migration. Beyond that, further experimental result displayed the reduction of H2BK5ac by Ras-ERK1/2 pathway might be realized via MDM2-mediated ATF2 degradation.

5. Conclusion

This work corroborated that Ras-ERK1/2 accelerated the progression of CRC through repression of H2BK5ac. HDAC2 and MDM2-mediated ATF2 degradation could affect the acetylation of H2BK5. These explorations offer a research foundation of Ras-ERK1/2 signaling and H2BK5ac in tumorigenesis, and also point out a neoteric avenues for remedying CRC.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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