P300-dependent acetylation of histone H3 is required for EGFR-mediated HMGA2 transcription in hepatocellular carcinoma

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Abstract

Background

High-mobility group protein A2 (HMGA2) is highly expressed in hepatocellular carcinoma cells and contributes to tumor metastasis and poor patient survival. However, the molecular mechanism of HMGA2 transcriptional regulation in hepatocellular carcinoma cells remains largely unclear.

Methods

Mouse model of lung metastasis was used to evaluate the effects of HMGA2 on hepatocellular carcinoma. DEN-induced rat model of hepatocellular carcinoma was used to measure the expression of HMGA2 and histone H3 acetylation. Immunohistochemistry was used to analyze the expression levels of HMGA2 in 39 matched HCC tissues and adjacent non-tumor tissues. Real-time PCR, Western blotting and Chromatin Immunoprecipitation assay were used to investigate the underlying mechanisms by which HMGA2 overexpression in hepatocellular carcinoma cells.

Result

Compared with adjacent non-tumour tissues, the expression of HMGA2 was significantly upregulated in human HCC tumor tissues. Moreover, We demonstrated that the expression HMGA2 was upregulated in HCC, and the elevated HMGA2 could promote tumor metastasis. Incubation HCC cells with Epidermal growth factor (EGF) could promote the expression of HMGA2 mRNA and protein. Knockdown of p300 can reverse EGF-induced HMGA2 expression and histone H3-K9 acetylation, while a phosphorylation-mimic p300 S1834D mutant can stimulate HMGA2 expression as well as H3-K9 acetylation in HCC cells. Furthermore, we identified p300-mediated H3-K9 acetylation participates in EGF-induced HMGA2 expression in HCC. In addition, the levels of H3-K9 acetylation positively correlated with the expression levels of HMGA2 in human HCC specimens.

Conclusion

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Background

Hepatocellular carcinoma (HCC) is one of the malignant tumors with poor prognosis, and ranked as the second common cause of death related to cancer[1]. The most important cause of death in HCC patients is the high metastatic capacity of hepatocellular carcinoma cell[2, 3]. Therefore, exploring the

mechanisms of hepatocellular carcinoma metastasis will be helpful for the prevention and treatment of hepatocellular carcinoma.

The high-mobility-group AT-hook protein 2 (HMGA2) is a kind of non-histone chromatin protein that is widely found in eukaryotes[4]. It contains three special at-hook structures, which can bind to at-sequence-enriched regions on DNA, promote or inhibit the expression of related target genes, and thus affect the development of embryos[5]. Generally, HMGA2 is only highly expressed at the early stage of fetal development, and the expression of HMGA2 can hardly be detected in normal adult tissues[6, 7]. A large number of studies have shown that HMGA2 is highly expressed in most malignant tumors, and its expression level is closely related to the degree of malignancy and metastasis, including breast, melanoma, colorectal and bladder cancer[8, 9]. More important, accumulating evidence have shown that HMGA2 is highly expressed in hepatocellular carcinoma, and the elevated HMGA2 predicts high incidence of metastasis and poor patient survival in hepatocellular carcinoma[10, 11]. For example, overexpression of HMGA2 is an important contributor to the invasion of hepatocellular carcinoma cells, while HMGA2 knockdown impaired the migratory capacity of tumor cells in hepatocellular carcinoma[12, 13]. However, the specific molecular mechanism of HMGA2 transcriptional regulation in hepatocellular carcinoma cells is still unclear.

Acetylation of histone H3 is one of the most frequent epigenetic modifications that affects chromatin structure and gene expression[14, 15]. It has been shown that histone deacetylase inhibition results in decreased HMGA2 transcription level and reduced HMGA2 promoter binding to acetylated histone H3 in HeLa cells[16]. This raised the possibility that histone H3 acetylation may be involved in regulating HMGA2 transcription in HCC.

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase and frequently overexpressed in hepatocellular carcinoma[17, 18]. Persistent activation of the EGFR signaling system has been implicated in liver responses to injury and carcinogenesis[19, 20]. Several studies have demonstrated that histone H3 acetylation in response to the activation of EGFR was associated with gene transcription and Tumorigenesis[21, 22]. However, it remains unclear whether EGFR-mediated H3 acetylation is involved in the transcription of HGMA2 in HCC. Here, our study demonstrated that EGFR activation induces p300 phosphorylation at S1834 via PI3K/Akt signaling pathway, then p300 can acetylate histone H3 at residue K9, leading to high expression of HMGA2 in hepatocellular carcinoma.

Materials And Methods

Reagents and antibodies

The inhibitors used in this study and the anti-HMGA2 antibody (#8179) were purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit polyclonal anti-Histone H3 antibody (ab1791), rabbit polyclonal anti-Histone H3 (acetyl K9) antibody (ab10812), mouse monoclonal anti-β-actin antibody (ab8226), horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (ab6721) and goat anti-mouse

IgG (ab205719) were all purchased from Abcam (Cambridge, MA, USA). Rabbit polyclonal anti-p300 (phosphor S1834) antibody (PA5-64531) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Besides, Recombinant Human EGF Protein (236-EG) was purchased from R&D systems (Minneapolis, MN, USA).

Cell lines and cell culture

SNU-368, SNU-739, Huh-7 and HepG2 cells were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (HyClone, Logan, UT, USA) at 37 °C in a humidified incubator containing 5% CO_2.

Western blot analysis

The cells or liver tissue were extracted using ice-chilled RIPA lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40, 5 mM EDTA, 0.25 mM PMSF and protease inhibitor cocktail. The concentration of protein was determined using the BCA kit (Pierce, Rockford, IL). Protein samples were denatured and separated by SDS-polyacrylamide gel electrophoresis. When the protein is completely separated, it is transferred to a methanol activated PVDF membrane. The membrane was then blocked in 5% skim milk powder or 3% BSA for 1 h at room temperature. After blocking, the PVDF membrane was then incubated with the corresponding primary and HRP-conjugated secondary antibodies. Finally, ECL plus reagent was used in the FluorChem E (Protein Simple, San Jose, CA, USA) imager to detect the blotting.

Real time PCR

RNA from cancer cells and rat liver tissues was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA was synthesized by using random hexamers and MMLV reverse transcriptase according to the manufacturer's instructions (Takara, Tokyo, Japan). Real-time quantitative PCR was performed using 2 × SYBR Green PCR Master Mix (Promega) on ABI7500 sequence detection system (Applied Biosystems). The setting procedures were as follows: 95℃, predenaturation for 5 min, 95℃ for 15 seconds, 60℃ for 1 minute, a total of 40 cycles. Phosphoglyceraldehyde dehydrogenase (GAPDH) was used as internal control. The relative expression level of HMGA2 was calculated by the $2^{-\Delta\Delta Ct}$ method. The primers sequences used were as follows: human HMGA2, 5'-TCCCTCTAAAGCAGCTCAAAA-3' (sense) and 5'-ACTTGTTGTGGCCATTTCCT-3' (antisense); human GAPDH, 5'-GACCCCTTCATTGACCTCAAC-3' (sense) and 5'-CTTCTCCATGGTGGTGAAGA-3' (antisense); rat HMGA2, 5'-ATCCCGTCTCCCGAAAGGT-3' (sense) and 5'-CTCGGTTGGACACTCGGGA-3' (antisense); rat GAPDH, 5'-GACAACTTTGGCATCGTGGA-3' (sense) and 5'-ATGCAGGGATGATGTTCTGG-3' (antisense).

Chromatin Immunoprecipitation

A chromatin immunoprecipitation (ChIP) assay was performed using the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) (#9004S, Cell Signaling Technology) according to the manufacturer's protocols. Primers flanking the acetyl-H3 binding sites on the promoters of HMGA2 were used for qRT-

PCR. Three replicates for each experimental point were performed. Results were normalized using the internal control IgG.

Animals and Experimental Design

Male Sprague-Dawley rats (6-week-old) and male athymic BALB/c nu/nu mice (5-week-old) were purchased from Beijing Weitong Lihua Animal Co. All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Henan University. During the experiment, all animals were housed in 20–24 °C with a relative humidity of 50 ± 10% and a light/dark cycle of 12 hours. The animals were fed with sufficient water and food every day. Before experimental procedures, all animals were allowed to adapt to the environment for a week.

Rat model of hepatocellular carcinoma was induced by diethylnitrosamine (DEN). Briefly, the rats were intraperitoneally injected with 50 mg/kg of diethylnitrosamine (DEN) once a week for 16 weeks. Rats in the control group were intraperitoneally injected with normal saline. At the end of the experiment, all animals were sacrificed and their livers were removed. Some livers were fixed with 4% paraformaldehyde and used for immunohistochemistry. The rest was stored at -80 °C for western blot and real time PCR.

In the lung metastasis experiment, we randomly divided BALB/c mice into 4 groups with 10 mice in each group: H22/sh-vector, H22/shHMGA2, H22/vector, H22/HMGA2-OE (HMGA2 overexpression). Then 1×10^6 cells transfected with the plasmids of vector, HMGA2 shRNA or HMGA2 overexpression were injected into the lateral caudal vein of each mouse. To ensure that the mice had prominent nodules in their lungs, pulmonary metastasis was allowed to develop for 6 weeks. Then the mice were sacrificed and their lungs were removed. After 1 day of fixation with 4% paraformaldehyde, the number of nodules on the lung surface of the mice in each group was counted.

Human tissue specimens

Between 2016 and 2018, we obtained matched HCC tissue and adjacent non-tumor tissues from 39 patients from the first affiliated hospital of Henan University. The use of patient tissues was approved by the hospital research ethics committee, and informed consent was obtained from all enrolled patients.

Immunohistochemical (IHC) staining

The tissues were dehydrated and embedded in paraffin after fixation with paraformaldehyde, and then cutting into pieces (4 μm) and placed on polylysine-coated slides. Immunohistochemical staining was incubated overnight with HGMA2 antibody (1:100) or acetyl-histone H3 antibody (1:100) at 4 °C overnight. Then the slides were washed 3 times with PBS and streptavidin-bound horseradish peroxidase antibody was incubated for 1 h at room temperature. Subsequently, DAB was used as chromogen and diaminobenzidine was developed. Immunoreactivity was examined under a light microscope. Two independent pathologists analyzed the expression of the target protein by visualizing the brown-stained section. The staining intensity was scored on a scale of 0–3: 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The staining extent was scored as follows: 0, if no tumour sections were stained; 1, if <1% of sections were stained; 2, if 2–10% of sections were stained; 3, if 11–30% of sections were stained; 4, 31–

70% of sections were stained; 5, 71–100% of sections were stained. The staining intensity and staining extent scores were then added to calculate the final stain score (0–8) for each tissue.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). All data are presented as means ± SEM. The statistical significance of the difference between the two groups was tested by an unpaired, two-tailed Student's t-test. One-way analysis of variance (ANOVA) followed by Tukey or Dunnet´s post-tests was used to compare means of multiple experimental groups. $p < 0.05$ was considered to be significant.

Results

HMGA2 is overexpressed and promotes tumor metastasis in hepatocellular carcinoma

Before exploring molecular mechanism of HMGA2 transcriptional regulation in hepatocellular carcinoma cells, we first collected 39 matched HCC tissue and adjacent non-tumour tissues and examined the expression of HMGA2. As the results of immunohistochemical staining (IHC) shown in Fig. 1A, the expression of HMGA2 in human HCC tumor tissues was higher than that in adjacent tissues. Moreover, we then established a hepatocellular carcinoma model by injecting diethylnitrosamine (DEN) into rats and detected the expression of HMGA2. Through real-time PCR and Western blotting, we found that the mRNA and protein expression of HMGA2 in the livers of DEN-treated rats were significantly up-regulated (Fig. 1B, C). The results of IHC further observed overexpression of HMGA2 protein in the livers of DEN-treated rat (Fig. 1D). In order to explore the role of HMGA2 in the metastasis of HCC, we established HMGA2-overexpressing and HMGA2-knockdown H22 cells. These cells were then inoculated into the lateral tail vein of BALB/c mice. After 6 weeks, we counted the lung metastases nodules. As the results shown, overexpression of HMGA2 increased the numbers of tumor metastatic nodules, while knockdown of HMGA2 significantly reduced the number of lung metastasis (Fig. 1E, F). Obviously, these results suggested that overexpression of HMGA2 in HCC can promote the metastasis of hepatocellular carcinoma.

EGF promotes HMGA2 transcription in hepatocellular carcinoma through the PI3K/AKT signaling pathways

Next, we investigated whether EGF promoted the expression of HMGA2. We stimulate SNU-368, SNU-739, Huh-7 and HepG2 cells with EGF, and detected the protein level of HMGA2. We found that the mRNA and protein expression levels of HMGA2 in HCC cells were significantly up-regulated after treatment with 100 ng/ml EGF (Fig. 2A-B). To determine whether EGF-induced HMGA2 overexpression is dependent on EGFR activation, we pretreated SNU-368 cells with 20 μM AG1478 (an inhibitor of EGFR tyrosine kinase) 30 min prior to co-incubation with 100 ng/ml EGF for 12 h. Through real time PCR and Western blot,we

found AG1478 could reverse the upregulation of HMGA2 mRNA and protein levels induced by EGF (Fig. 2C, D).

EGFR carries out its biological functions through activation of the Src-JAK-STAT3, Ras-Raf-MEK-ERK, and PI3K-Akt-mTOR pathways[17, 23]. To explore the pathways by which EGF induces HMGA2 expression in HCC cells, we examined the effects of AG490 (20 μM, a JAK inhibitor), PD98059 (20 μM, a MEK inhibitor), and MK2206 (10 μM, an Akt inhibitor) on EGF-induced HMGA2 mRNA and protein expression in HCC cells. As the results shown, pretreatment with MK2206 can reverse EGF-induced HMGA2 mRNA and protein expression in SNU-368 cells (Fig. 2C, D) after co-incubation with 100 ng/ml EGF for 12 h, while AG490 and PD98059 had no significant effect on EGF-induced HMGA2 mRNA and protein expression (Fig. 2E, F). These results clearly demonstrate that EGF promoted HMGA2 expression through PI3K/Akt pathways.

Akt-dependent p300 phosphorylation mediates the EGF-induced increase in HMGA2 transcription in HCC

Previous literature has shown that EGFR phosphorylates the p300 S1834 residue through the PI3K/AKT pathway, thereby promoting the expression of the relevant gene[24]. Therefore, we then investigated whether phosphorylation of p300 is involved in EGF-induced HMGA2 transcriptional expression. Firstly, we incubated SNU-368 cells using EGF and detected the phosphorylation level on p300 ser1834 residues. As the results shown in Fig. 3A, after exposure of SNU-368 cells to 100 ng/ml EGF, the phosphorylation level of p300 S1834 residues in SNU-368 cells was significantly increased. Then, the effects of AG1478 (an inhibitor of EGFR tyrosine kinase) and MK2206 (10 μM, an Akt inhibitor) on EGF-induced phosphorylation of p300 S1834 residues were evaluated in SNU-368 cells, respectively. The results showed that when SNU-368 cells were pretreated with AG1478 or MK2206 for 30 minutes followed by coincubation with 100 ng/mL EGF for 2 h, EGF-induced increase in the phosphorylation of p300 S1834 can be reversed by both of these two inhibitors (Fig. 3B). These data indicate that EGF phosphorylates p300 at S1834 site via PI3K/ Akt signaling pathway in HCC.

Subsequently, we examined whether p300 participates in EGF-induced upregulation of HMGA2 expression in HCC. As the results shown, knockdown of P300 with shRNA could reverse EGF-enhanced HMGA2 mRNA and protein in SNU-368 cells (Fig. 3C, D). To verify that p300 phosphorylation at S1834 site is involved in EGF-induced expression of HMGA2 protein and mRNA levels, we constructed a Flag-tagged WT p300, a phosphorylation-defective p300 S1834A mutant and a phosphorylation-mimic p300 S1834D mutant was transfected them into SNU-368 cells. After transfection for 24 h, we detected the expression HMGA2 mRNA and protein. The results showed that only a phosphorylation-mimic p300 S1834D mutant can stimulate HMGA2 mRNA and protein expression in SNU-368 cells (Fig. 3E, F).

P300-dependent acetylation of histone H3 K9 is required for EGFR-mediated HMGA2 transcription in hepatocellular carcinoma

Acetylation of histone H3 K9 in response to EGFR activation was associated with gene expression and tumorigenesis[22]. As an acetyltransferase, p300 can enhance the acetylation of histones H3 K9, leading to the activation of target gene expression[25, 26]. Therefore, we supposed that p300-dependent acetylation of histone H3 K9 is required for EGFR-mediated HMGA2 transcription in hepatocellular carcinoma. To test this hypothesis, we firstly stimulated SNU-368 cells with EGF to see if it could induce the acetylation of histone H3 K9. As shown in Fig. 4A, the acetylation level at K9 site was significantly up-regulated when EGF was applied to SNU-368 cells. Then, we knockdown p300 using shRNA and examined the level of H3 K9 acetylation induced by EGF treatment. As expected, knockdown of p300 can attenuate EGF-induced increase level of H3 K9 acetylation in SNU-368 cells (Fig. 4B). Moreover, we also transfected a Flag-tagged WT p300, a phosphorylation-defective p300 S1834A mutant and a phosphorylation-mimic p300 S1834D mutant into SNU-368 cells and examined the level of histones H3 K9 acetylation. As the results shown in Fig. 4C, only a phosphorylation-mimic p300 S1834D mutant can stimulate histones H3 K9 acetylation in SNU-368 cells. Together, these results suggested that histones H3 K9 acetylation depends on EGF-mediated p300 phosphorylation at S1834 site.

To explore the significance of histone H3-K9 acetylation in EGF-induced HMGA2 expression, we replaced histone K9 site with arginine to construct the H3 K9R mutant and expressed it into endogenous H3-depleted SNU-368 cells to detect the role of H3-K9 acetylation in EGF-induced HMGA2 expression. As the results shown, Mutation of K9 of histone H3 into arginine abrogated EGF-induced mRNA and protein expression of HMGA2 (Fig. 4D, E). Meanwhile, chromatin immunoprecipitation results also showed that EGF stimulation can lead to increased acetylation of H3 K9 at HMGA2 promoter in SNU-368 cells (Fig. 4F). In vivo experiments, we detected the acetylation level of histone H3 K9 in DEN-induced rat liver tissues using Western blot and immunohistochemical staining. Through these two methods, we observed an enhanced acetylation level of histone H3 K9 in the livers of DEN-treated rats (Fig. 5A, B). Subsequently, we performed ChIP assay in the livers of DEN-treated rats and normal rats. Using an acetyl-H3-K9 antibody, we found DEN administration resulted in an enhanced H3-K9 acetylation at the HMGA2 promoter in the rat livers (Fig. 5C). To define the clinical relevance of our finding that H3-K9 acetylation regulates HMGA2 transcription, we performed immunohistochemistry analyses of 39 human HCC tumor specimens with antibodies against H3-K9 acetylation and HMGA2. As the results shown, the levels of H3-K9 acetylation correlated with the expression levels of HMGA2, quantification of the staining showed that these correlations were significant(Fig. 5D, E). Taken together, these results suggest p300-dependent acetylation of histone H3 K9 is necessary for EGF-induced HMGA2 expression in HCC.

Discussion

Overexpression of HMGA2 is present in many epithelial malignancies, such as pancreatic cancer, breast cancer, ovarian cancer, oral squamous cell carcinoma and lung cancer[8]. Accumulating evidences have suggested HMGA2 functions as a tumor promoter, which can promote tumor metastasis and enhance the formation of Epithelial-mesenchymal transition (EMT)[12, 27]. For example, HMGA2 can induce enhanced transcription of Twist1 and Snail by directly binding to the critical element of endogenous target promoters, resulting in EMT[28, 29]; the silencing of HMGA2 expression inhibited glioblastoma invasion

via repression of the transcription of MMP2 gene[30]. Similarly, highly expressed HMGA2 was also observed in hepatocellular carcinoma cells, and the elevated expression of HMGA2 could promote hepatocellular carcinoma cell migration and invasion[10, 12]. In support of these studies, we also observed an abnormal expression of HMGA2 in the livers of DEN-treated rats; overexpression of HMGA2 increased the numbers of tumor metastatic nodules in a mouse model of lung metastasis.

Recently, several lines of reports focused on the regulation of HMGA expression have emerged. For example, long noncoding RNA VPS9D1 antisense RNA 1 (VPS9D1-AS1) promotes HMGA2 expression by sponging miR-532-3p in non-small cell lung cancer (NSCLC) cells[31]. Oxidized low density lipoprotein receptor 1 (OLR1) increased HMGA2 transcription by upregulating c-Myc expression in pancreatic cancer cells[32]. However, the mechanism underlying which regulates the transcriptional expression of HMGA2 in hepatocellular carcinoma cells remains largely unclear. Here, our findings suggested EGF stimulation can promotes HMGA2 expression at transcriptional level via activation of PI3K/Akt signaling pathways in hepatocellular carcinoma cells. In support of our results, Voon DC et al found exposure murine gastric epithelial (GIF-14) cells to EGF led to an increased HMGA2 transcription[33]. Certainly, we cannot rule out other mechanisms are involved in the regulation of HMGA2 expression in HCC. In fact, several long non-coding RNAs (lncRNAs), such as HULC, LSINCT5, LINC00473, SNHG16 and EGOT, have been found to promote HMGA2 expression in HCC[11, 34–37]. In addition, a series of microRNAs (miRs or miRNAs) including miR-663a, miR-107, microRNA-337 and microRNA-9 could inhibit hepatocellular carcinoma progression through suppression of HMGA2 expression[38–40]. However, whether these lncRNAs or microRNAs could interact with EGF and have a synergistic effect in regulation of HMGA2 transcription needs to be further explored.

Abnormal expression of p300 has been identified in esophageal squamous carcinoma, NSCLC cells and hepatocellular carcinoma[41–43]. Numerous studies have demonstrated p300 was associated with enhanced EMT and correlates with poor prognosis in these cancers[41, 43]. Overexpression of p300 promotes cell proliferation, migration, and invasion in NSCLC cells[42]; knockdown of p300 inhibited EMT, invasion and other malignant events of HCC cells[43]. However, we must note that p300 may act as a suppressor of tumor metastasis in some cancers. For example, Wang Y et al have reported that p300 can acetylate JHDM1A to inhibit the growth and metastasis of osteosarcoma[44]. Act as a transcriptional coactivator, p300 performs these functions through altering chromatin structure to stimulate tumor-related genes transcription[45, 46]. In detail, p300 controls these genes expression by transferring an acetyl group to lysine residues on histones and non-histone proteins[45]. Consistent with these reports, we demonstrated that p300 could acetylate histone H3 at K9 and promote HMGA2 transcription in hepatocellular carcinoma. To the best of our knowledge, we disclosed for the first time that p300 can stimulate HMGA2 expression at transcriptional level. As an acetyltransferase, the catalytic activity of p300 can be regulated by post-translational modification including methylation, acetylation as well as phosphorylation at some critical sites[47]. For instance, a wide array of protein kinases, such as protein kinase A (PKA), PKB, PKC, and mitogen-activated protein kinases (MAPK) can catalyze p300 phosphorylation at specific sites, resulting in activation or repression of target gene expression[47, 48]. Here, we also demonstrated that Akt could phosphorylate p300 at S1834 site to enhance the activity of

p300, which promotes HMGA2 transcription in hepatocellular carcinoma cells. In support of our findings, Srivastava S et al also demonstrated Akt could directly phosphorylate p300 at S1834 in response to EGF stimulation in lung cancer cells.

Histone H3 acetylation has been shown to participate in the regulation of transcriptional activity by altering the structure of the chromatin[49]. In generally, hyperacetylation is largely associated with the activation of gene transcription, while hypoacetylation means repression of gene expression. Altered levels of acetylation of H3 at lysine (K) 4, 9, 14, 27 have been correlated with tumor progression and metastasis in a variety of cancers[50]. Although our results demonstrated H3 K9 acetylation plays an important role in promoting HMGA2 transcription, we could not exclude role of other lysine residues in regulation of HMGA2 gene expression, because several studies have noted that K4, K14 and K27 can be acetylated upon EGF stimulation[21, 51]. Histone H3 acetylation can be achieved by two groups of enzymes with opposing functions: histone acetyltransferases (HATs) and histone deacetylases (HDACs) [49]. Here we identified that p300, one member of HAT family, can add acetyl moieties to K9 residues upon EGFR activation in HCC. However, we cannot rule out the possibility that other HATs or HDACs are involved in catalyzing H3 K9 acetylation.

Conclusions

We first revealed that EGF can induce p300 phosphorylation at Ser1834 site through activation of PI3K/AKT signaling pathway in hepatocellular carcinoma, p300 subsequently catalyzes histone H3 acetylation at K9 residues, leading to HMGA2 transcription in HCC (Fig. 6).

Abbreviations

HMGA2: High-mobility group protein A2; EGF:Epidermal growth factor; HCC:Hepatocellular carcinoma; EGFR:Epidermal growth factor receptor; DMEM:Dulbecco's modified Eagle's medium;DEN□Diethylnitrosamine; HMGA2-OE:HMGA2 overexpression; IHC:Immunohistochemical; ChIP:Chromatin Immunoprecipitation; ANOVA:One-way analysis of variance; HMGA2-KD:HMGA2-knockdown; Ac-H3:Acetylation of histone H3; EMT:Epithelial-mesenchymal transition; NSCLC:non-small cell lung cancer; OLR1:Oxidized low density lipoprotein receptor 1; lncRNAs:long non-coding RNAs; miRNAs:microRNAs; PKA:protein kinase A; MAPK:mitogen-activated protein kinases; HATs:histone acetyltransferases; HDACs:histone deacetylases.

Declarations

Availability of data and materials

All experimental data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

All experiments regarding human tissue slides and animal study in this study were performed in according to ethical standards. Informed consent was obtained from all patients and animal study was approved by the Animal Center of Henan University.

Consent for publication

All authors involved in the study had given their consent for submitting this article for publication.

Competing interests

The authors declare that they have no competing interest.

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Author contributions

C.L., X.W., performed Western blot analysis and real time PCR. X.Y., R.Y., Z.Z. performed all animal experiments. F.L and S.P. carried out Immunoprecipitation and Chromatin Immunoprecipitation. H.S. participated in the data analysis. D.F., S.X. participated in the design of the study and manuscript writing. All authors read and approved the final manuscript.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68(6):394–424.
2. Dutta R, Mahato RI. Recent advances in hepatocellular carcinoma therapy. Pharmacol Ther. 2017;173:106–17.
3. Giannelli G, Koudelkova P, Dituri F, Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. Journal of hepatology. 2016;65(4):798–808.

4. Pfannkuche K, Summer H, Li O, Hescheler J, Droge P. The high mobility group protein HMGA2: a co-regulator of chromatin structure and pluripotency in stem cells? Stem cell reviews reports. 2009;5(3):224–30.
5. Hammond SM, Sharpless NE. HMGA2, microRNAs, and stem cell aging. Cell 2008, 135(6):1013–1016.
6. Vignali R, Marracci S. HMGA Genes and Proteins in Development and Evolution. International journal of molecular sciences 2020, 21(2).
7. Parisi S, Piscitelli S, Passaro F, Russo T. HMGA Proteins in Stemness and Differentiation of Embryonic and Adult Stem Cells. International journal of molecular sciences 2020, 21(1).
8. Zhang S, Mo Q, Wang X. Oncological role of HMGA2 (Review). Int J Oncol. 2019;55(4):775–88.
9. Sgarra R, Pegoraro S, Ros G, Penzo C, Chiefari E, Foti D, Brunetti A, Manfioletti G. High Mobility Group A (HMGA) proteins: Molecular instigators of breast cancer onset and progression. Biochimica et biophysica acta Reviews on cancer. 2018;1869(2):216–29.
10. Zhao YC, Jiao Y, Li YQ, Fu Z, Yang ZY, He M. Elevated high mobility group A2 expression in liver cancer predicts poor patient survival. Revista espanola de enfermedades digestivas: organo oficial de la Sociedad Espanola de Patologia Digestiva 2020, 112(1):27–33.
11. Li S, Peng F, Ning Y, Jiang P, Peng J, Ding X, Zhang J, Jiang T, Xiang S. SNHG16 as the miRNA let-7b-5p sponge facilitates the G2/M and epithelial-mesenchymal transition by regulating CDC25B and HMGA2 expression in hepatocellular carcinoma. Journal of cellular biochemistry. 2020;121(3):2543–58.
12. Luo Y, Li W, Liao H. HMGA2 induces epithelial-to-mesenchymal transition in human hepatocellular carcinoma cells. Oncology letters. 2013;5(4):1353–6.
13. Hengjuan LV, Liu G, Kun LI, Mingqiu LI, Zhang D. Angiogenin regulates epithelial-mesenchymal transition of hepatocellular carcinoma through upregulation of HMGA2. Pharmazie. 2019;74(5):301–4.
14. Pradeepa MM, Grimes GR, Kumar Y, Olley G, Taylor GC, Schneider R, Bickmore WA. Histone H3 globular domain acetylation identifies a new class of enhancers. Nat Genet. 2016;48(6):681–6.
15. Guo P, Chen W, Li H, Li M, Li L. The Histone Acetylation Modifications of Breast Cancer and their Therapeutic Implications. Pathology oncology research: POR. 2018;24(4):807–13.
16. Ferguson M, Henry PA, Currie RA. Histone deacetylase inhibition is associated with transcriptional repression of the Hmga2 gene. Nucleic acids research. 2003;31(12):3123–33.
17. Huang P, Xu X, Wang L, Zhu B, Wang X, Xia J. The role of EGF-EGFR signalling pathway in hepatocellular carcinoma inflammatory microenvironment. J Cell Mol Med. 2014;18(2):218–30.
18. Ye QH, Zhu WW, Zhang JB, Qin Y, Lu M, Lin GL, Guo L, Zhang B, Lin ZH, Roessler S, et al. GOLM1 Modulates EGFR/RTK Cell-Surface Recycling to Drive Hepatocellular Carcinoma Metastasis. Cancer cell. 2016;30(3):444–58.

19. Berasain C, Avila MA. The EGFR signalling system in the liver: from hepatoprotection to hepatocarcinogenesis. Journal of gastroenterology. 2014;49(1):9–23.
20. Komposch K, Sibilia M. EGFR Signaling in Liver Diseases. International journal of molecular sciences 2015, 17(1).
21. Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Molecular cell. 2000;5(6):905–15.
22. Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D, Aldape K, Hunter T, Alfred Yung WK, Lu Z. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell. 2012;150(4):685–96.
23. Li W, Hou JZ, Niu J, Xi ZQ, Ma C, Sun H, Wang CJ, Fang D, Li Q, Xie SQ. Akt1 inhibition promotes breast cancer metastasis through EGFR-mediated beta-catenin nuclear accumulation. Cell communication signaling: CCS. 2018;16(1):82.
24. Srivastava S, Mohibi S, Mirza S, Band H, Band V. Epidermal Growth Factor Receptor activation promotes ADA3 acetylation through the AKT-p300 pathway. Cell cycle. 2017;16(16):1515–25.
25. Zhou W, Jiang D, Tian J, Liu L, Lu T, Huang X, Sun H. Acetylation of H3K4, H3K9, and H3K27 mediated by p300 regulates the expression of GATA4 in cardiocytes. Genes diseases. 2019;6(3):318–25.
26. Qiao Y, Wang R, Yang X, Tang K, Jing N. Dual roles of histone H3 lysine 9 acetylation in human embryonic stem cell pluripotency and neural differentiation. J Biol Chem. 2015;290(16):9949.
27. Hawsawi O, Henderson V, Burton LJ, Dougan J, Nagappan P, Odero-Marah V. High mobility group A2 (HMGA2) promotes EMT via MAPK pathway in prostate cancer. Biochem Biophys Res Commun. 2018;504(1):196–202.
28. Sun J, Sun B, Sun R, Zhu D, Zhao X, Zhang Y, Dong X, Che N, Li J, Liu F, et al. HMGA2 promotes vasculogenic mimicry and tumor aggressiveness by upregulating Twist1 in gastric carcinoma. Scientific reports. 2017;7(1):2229.
29. Tan EJ, Thuault S, Caja L, Carletti T, Heldin CH, Moustakas A. Regulation of transcription factor Twist expression by the DNA architectural protein high mobility group A2 during epithelial-to-mesenchymal transition. J Biol Chem. 2012;287(10):7134–45.
30. Zhang S, Zhang H, Yu L. HMGA2 promotes glioma invasion and poor prognosis via a long-range chromatin interaction. Cancer medicine 2018.
31. Han X, Huang T, Han J. Long noncoding RNA VPS9D1-AS1 augments the malignant phenotype of non-small cell lung cancer by sponging microRNA-532-3p and thereby enhancing HMGA2 expression. Aging. 2020;12(1):370–86.
32. Yang G, Xiong G, Feng M, Zhao F, Qiu J, Liu Y, Cao Z, Wang H, Yang J, You L, et al. OLR1 Promotes Pancreatic Cancer Metastasis via Increased c-Myc Expression and Transcription of HMGA2. Molecular cancer research: MCR; 2020.

33. Voon DC, Wang H, Koo JK, Chai JH, Hor YT, Tan TZ, Chu YS, Mori S, Ito Y. EMT-induced stemness and tumorigenicity are fueled by the EGFR/Ras pathway. PloS one. 2013;8(8):e70427.
34. Wang Y, Chen F, Zhao M, Yang Z, Li J, Zhang S, Zhang W, Ye L, Zhang X. The long noncoding RNA HULC promotes liver cancer by increasing the expression of the HMGA2 oncogene via sequestration of the microRNA-186. J Biol Chem. 2017;292(37):15395–407.
35. Jiang H, Li Y, Li J, Zhang X, Niu G, Chen S, Yao S. Long noncoding RNA LSINCT5 promotes endometrial carcinoma cell proliferation, cycle, and invasion by promoting the Wnt/beta-catenin signaling pathway via HMGA2. Therapeutic advances in medical oncology. 2019;11:1758835919874649.
36. Mo J, Li B, Zhou Y, Xu Y, Jiang H, Cheng X, Wu X, Zhang Y: LINC00473 promotes hepatocellular carcinoma progression via acting as a ceRNA for microRNA-195 and increasing HMGA2 expression. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2019, 120:109403.
37. Wu S, Ai H, Zhang K, Yun H, Xie F. Long Non-Coding RNA EGOT Promotes the Malignant Phenotypes of Hepatocellular Carcinoma Cells and Increases the Expression of HMGA2 via Down-Regulating miR-33a-5p. OncoTargets therapy. 2019;12:11623–35.
38. Huang W, Li J, Guo X, Zhao Y, Yuan X. miR-663a inhibits hepatocellular carcinoma cell proliferation and invasion by targeting HMGA2. Biomedicine pharmacotherapy = Biomedecine pharmacotherapie. 2016;81:431–8.
39. Wang Y, Chen F, Zhao M, Yang Z, Zhang S, Ye L, Gao H, Zhang X. MiR-107 suppresses proliferation of hepatoma cells through targeting HMGA2 mRNA 3'UTR. Biochem Biophys Res Commun. 2016;480(3):455–60.
40. Xu X, Zou H, Luo L, Wang X, Wang G. MicroRNA-9 exerts antitumor effects on hepatocellular carcinoma progression by targeting HMGA2. FEBS open bio. 2019;9(10):1784–97.
41. Bi Y, Kong P, Zhang L, Cui H, Xu X, Chang F, Yan T, Li J, Cheng C, Song B, et al. EP300 as an oncogene correlates with poor prognosis in esophageal squamous carcinoma. J Cancer. 2019;10(22):5413–26.
42. Hou X, Gong R, Zhan J, Zhou T, Ma Y, Zhao Y, Zhang Y, Chen G, Zhang Z, Ma S, et al. p300 promotes proliferation, migration, and invasion via inducing epithelial-mesenchymal transition in non-small cell lung cancer cells. BMC Cancer. 2018;18(1):641.
43. Ma C, Huang S, Xu L, Tian L, Yang Y, Wang J. Transcription co-activator P300 activates Elk1-aPKC-iota signaling mediated epithelial-to-mesenchymal transition and malignancy in hepatocellular carcinoma. Oncogenesis. 2020;9(3):32.
44. Wang Y, Sun B, Zhang Q, Dong H, Zhang J. p300 Acetylates JHDM1A to inhibit osteosarcoma carcinogenesis. Artificial cells nanomedicine biotechnology. 2019;47(1):2891–9.
45. Wang F, Marshall CB, Ikura M. Transcriptional/epigenetic regulator CBP/p300 in tumorigenesis: structural and functional versatility in target recognition. Cell Mol Life Sci. 2013;70(21):3989–4008.
46. Lasko LM, Jakob CG, Edalji RP, Qiu W, Montgomery D, Digiammarino EL, Hansen TM, Risi RM, Frey R, Manaves V, et al. Discovery of a selective catalytic p300/CBP inhibitor that targets lineage-specific tumours. Nature. 2017;550(7674):128–32.

47. Ghosh AK, Varga J. The transcriptional coactivator and acetyltransferase p300 in fibroblast biology and fibrosis. Journal of cellular physiology. 2007;213(3):663–71.
48. Vo N, Goodman RH. CREB-binding protein and p300 in transcriptional regulation. J Biol Chem. 2001;276(17):13505–8.
49. Audia JE, Campbell RM. Histone Modifications and Cancer. Cold Spring Harb Perspect Biol. 2016;8(4):a019521.
50. Kurdistani SK. Histone modifications as markers of cancer prognosis: a cellular view. British journal of cancer. 2007;97(1):1–5.
51. Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W, Gao X, Aldape K, Lu Z. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature. 2011;480(7375):118–22.

Figures

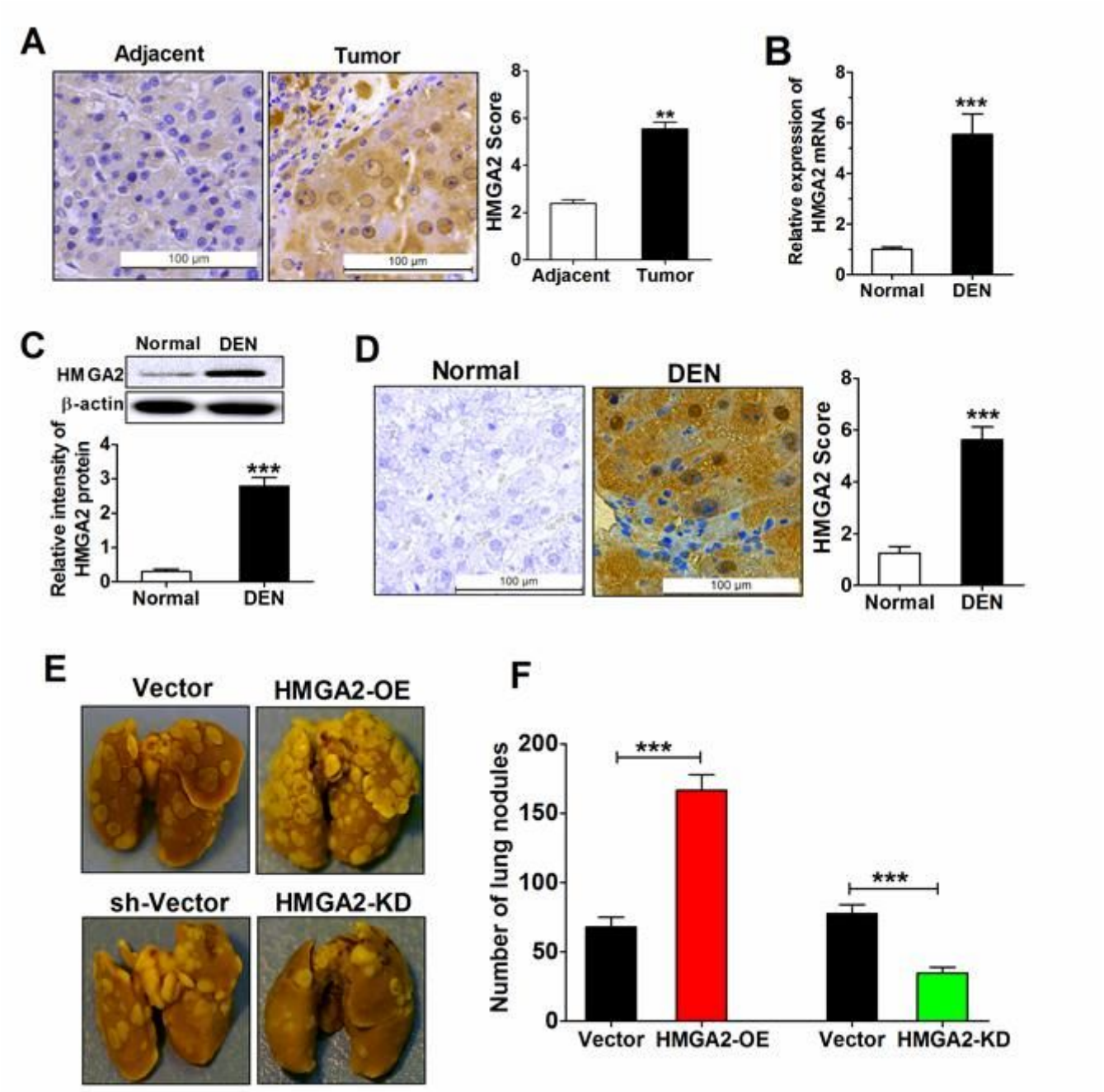

Figure 1

HMGA2 is upregulated and promotes tumor metastasis in hepatocellular carcinoma. (A) Immunohistochemical staining suggested that HMGA2 expression was increased in tumor tissues compared to adjacent non-tumor tissues, **p < 0.01, a two-tailed unpaired t-test, n = 39 per group. (B) The real time PCR assay showed that the mRNA expression of HMGA2 was upregulated in the livers of DEN-treated rats. ***p < 0.001 compared to normal rats, a two-tailed unpaired t-test, n = 8 per group. (C) Western blot analysis showed that the protein expression of HMGA2 was upregulated in the livers of DEN-treated rats. ***p < 0.001 compared to normal rats, a two-tailed unpaired t-test, n = 6 per group. (D) Immunohistochemical staining suggested that HMGA2 expression in the livers of DEN-rats was enhanced compared to normal rats, ***p < 0.001, a two-tailed unpaired t-test, n = 8 per group. (E, F) HMGA2 was

involved in promoting HCC metastasis. (E): representative photos of metastatic lung nodules from vector, HMGA2-overexpression (HMGA2-OE) and HMGA2-knockdown (HMGA2-KD) groups; (F): quantification of the lung nodules. Note that overexpression of HMGA2 increased the number of tumor metastatic nodules, while knockdown of HMGA2 significantly reduced the number of lung metastases in tumor-bearing mice. ***p < 0.001, a two-tailed unpaired t-test, n = 10 per group.

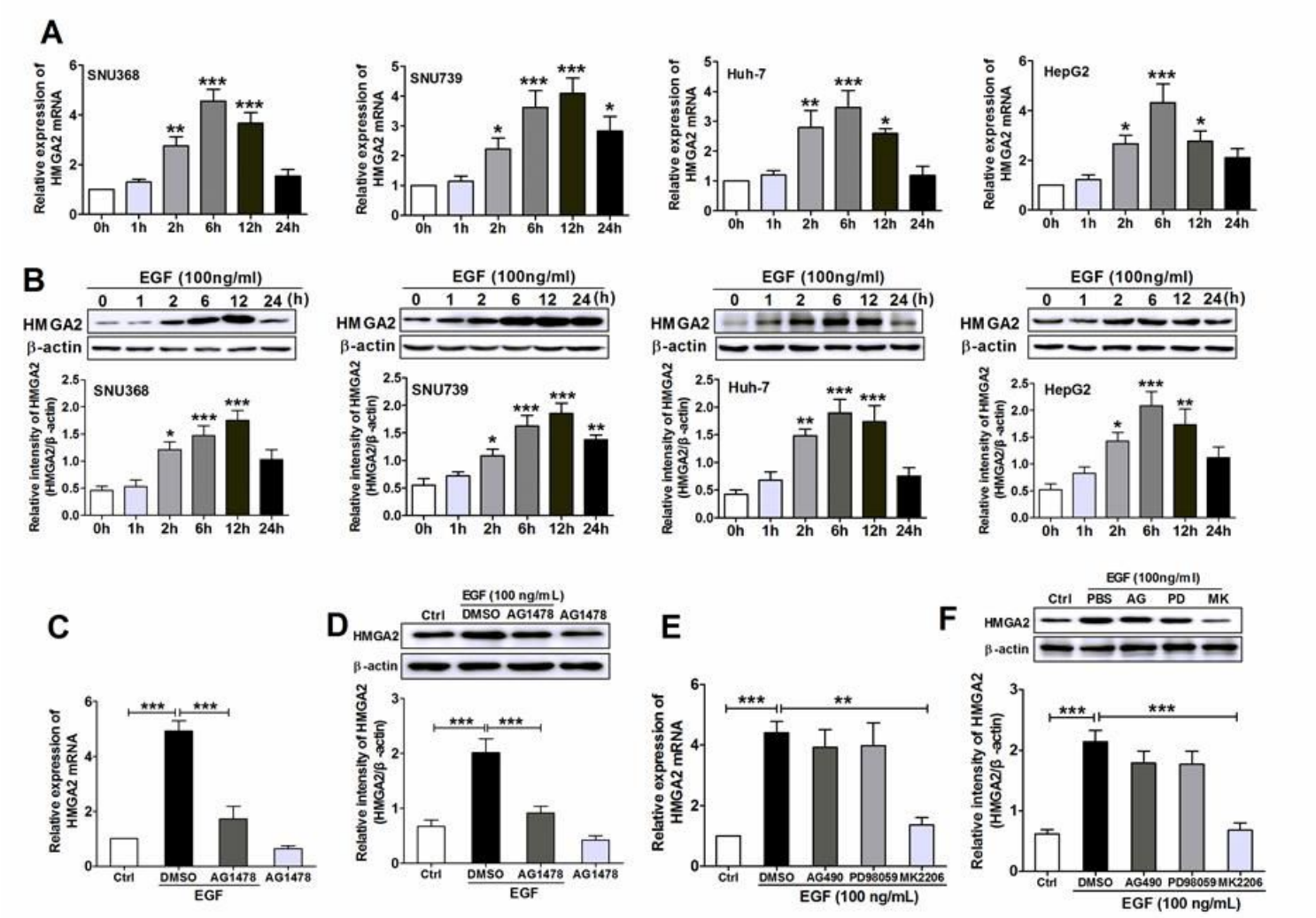

Figure 2

EGF promotes HMGA2 expression through PI3K/AKT signaling pathway in hepatocellular carcinoma. (A, B) EGF induced a significant increase in HMGA2 mRNA (A) and protein expression (B) in SNU-368, SNU-739, Huh-7 and HepG 2 cells. *p < 0.05, **p < 0.01, ***p < 0.001 compared to control group, one-way ANOVA, n = 6 per group. (C, D) EGFR tyrosine kinase inhibitor AG1478 (20 μM) could reverse EGF-mediated the upregulation of HMGA2 mRNA (C) and protein (D) expression in SNU-368 cells. ***p < 0.001 compared to control group, one-way ANOVA, n = 6 per group. (E, F) EGF-induced upregulation of HMGA2 mRNA (E) and protein (F) can be blocked by Akt inhibitor MK2206 (10 μM) in SNU-368 cells. While JAK inhibitor AG490 (20 μM) and MEK inhibitor PD98059 (20 μM) have no effects on EGF-induce HMGA2 mRNA and protein expression. ***p < 0.001, one-way ANOVA, n = 6 per group.

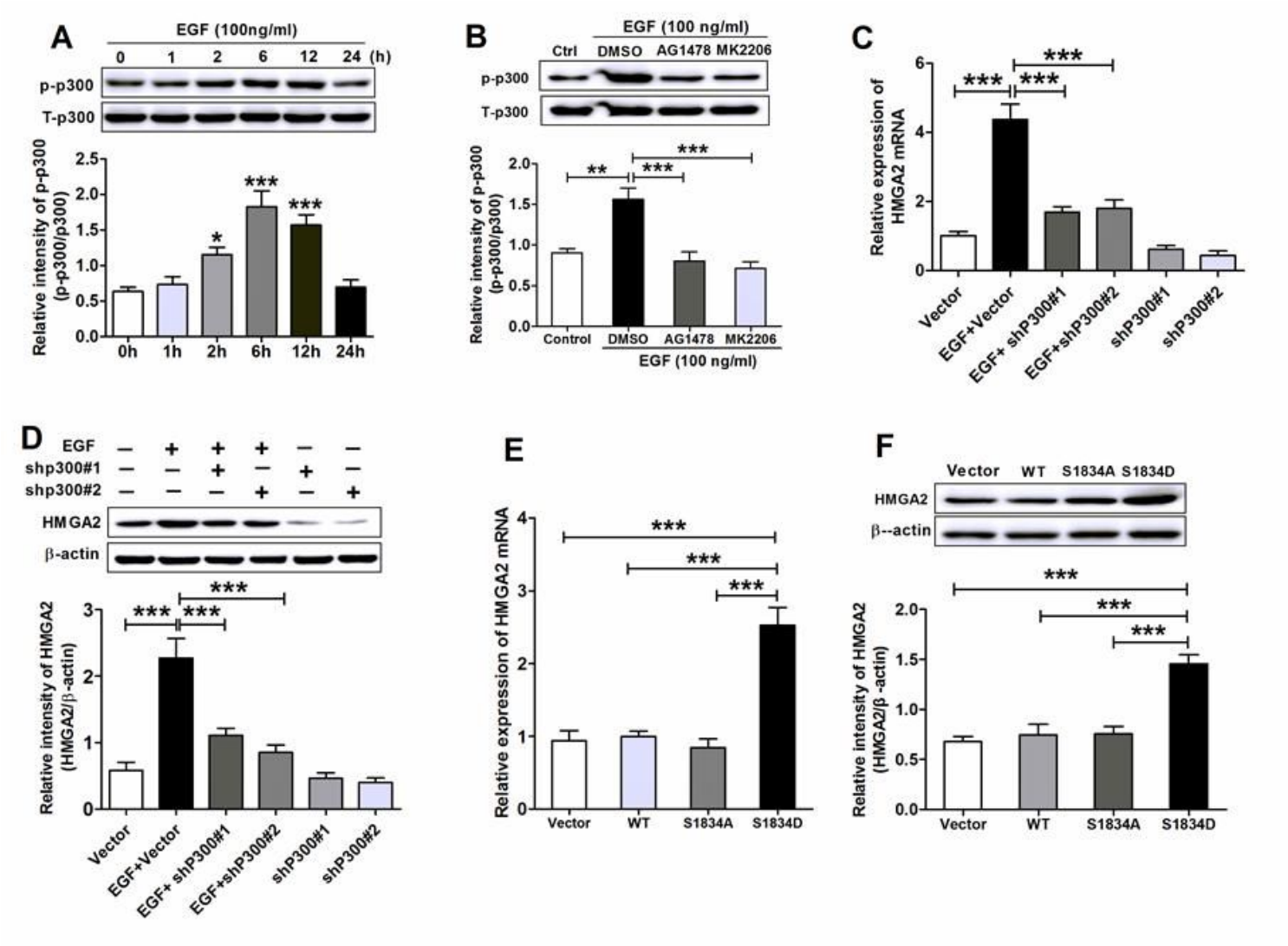

Figure 3

Akt-dependent p300 phosphorylation mediates the EGF-induced increase in HMGA2 transcription in SNU-368 cells. (A) Western blot analysis suggested that EGF induced an increase in the level of p300 S1834 phosphorylation in SNU-368 cells. *p < 0.05, ***p < 0.001, one-way ANOVA, n = 6 per group. (B) EGF-induced increased phosphorylation levels of p300 at S1834 was attenuated by 20 μM AG1478 (an EGFR tyrosine kinase inhibitor) and MK2206 (an AKT inhibitor) after 2 h co-incubation in SNU-368 cells. **p < 0.01, ***p < 0.001, one-way ANOVA, n = 5 per group. (C, D) Knockdown of p300 with two specific shRNAs could reverse EGF-induced HMGA2 mRNA (C) and protein (D) expression in SNU-368 cells, ***p < 0.001, one-way ANOVA, n = 6 per group. (E, F) Expression of a phosphorylation-mimic p300 S1834D mutant could induce HMGA2 mRNA (E) and protein (F) in SNU-368 cells. ***p < 0.001, one-way ANOVA, n = 6 per group.

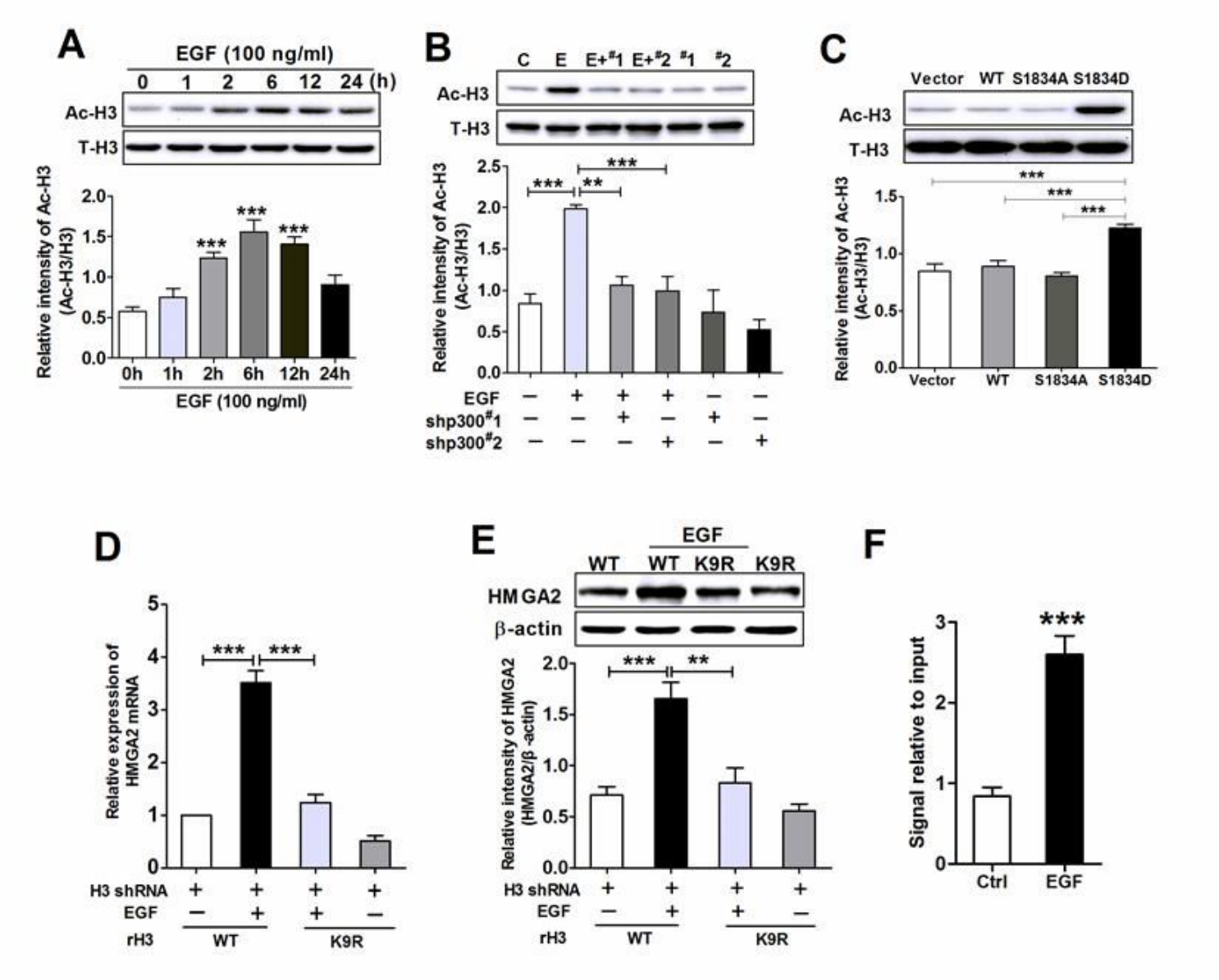

Figure 4

p300-dependent acetylation of histone H3 is required for EGFR-mediated HMGA2 transcription in SUN-368 cells. (A) EGF treatment induced a significant increase in H3-K9 acetylation in SNU-368 cells, **p < 0.01, ***p < 0.001, one-way ANOVA, n = 6 per group. Knockdown of p300 reversed EGF-induced H3-K9 acetylation in SNU-368 cells. C: control, E: EGF, #1: shp300#1, #2: shp300#2, ***p < 0.001, one-way ANOVA, n = 5 per group. (B) P300 knockdown could reverse EGF-induced H3-K9 acetylation in SNU-368 cells, **p < 0.01, ***p < 0.001, one-way ANOVA, n = 5 per group. (C) Expression of the phosphorylation-mimic p300 S1834D mutant induced a higher expression of H3-K9 acetylation in SNU-368 cells. ***p < 0.001, one-way ANOVA, n = 5 per group. (D, E) Reconstituted expression of RNAi-resistant histone H3-K9R abrogated EGF-induced the mRNA (D) and protein (E) expression of HMGA2 in endogenous H3-depleted SNU-368 cells. **p < 0.01, ***p < 0.001, one-way ANOVA, n = 5 per group. (F) ChIP analysis showed that EGF treatment resulted in enhanced H3-K9 acetylation at the HMGA2 promoter in SNU-368 cells. **p < 0.01, a two-tailed unpaired t-test, n = 5 per group.

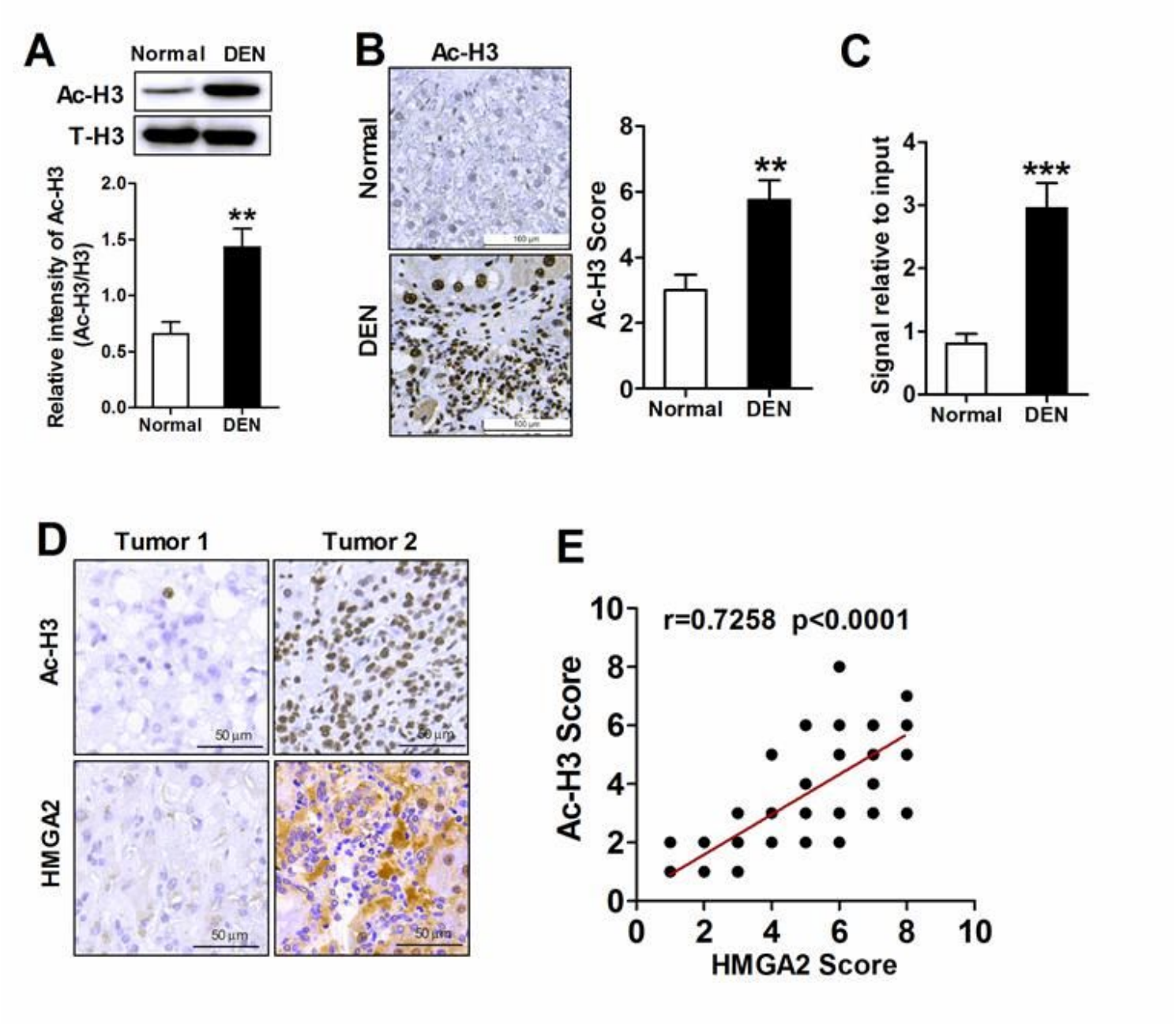

Figure 5

Histone H3 K9 acetylation is positively correlated with HMGA2 expression in HCC. (A) Western blot analysis showed that acetyl-H3 K9 expression was upregulated in the livers of DEN-treated rats. **p < 0.01 compared to normal rats, a two-tailed unpaired t-test, n = 8 per group. (B) Immunohistochemical staining suggested that acetyl-H3 expression in the livers of DEN-rats was enhanced compared to normal rats, **p < 0.01, a two-tailed unpaired t-test, n = 8 per group. (C) ChIP analyses with an acetyl-H3-K9 antibody demonstrated that DEN administration resulted in an enhanced H3-K9 acetylation at the HMGA2 promoter in rats. ***p < 0.001, a two-tailed unpaired t-test, n = 8 per group. (D) Representative photos of immunohistochemical staining of 39 HCC specimens with anti-acetyl-H3-K9 and HMGA2 antibodies. (E) The levels of H3-K9 acetylation correlated with the expression levels of HMGA2. Pearson product moment

correlation test, note that some of the dots on the graphs represent more than one specimen (i.e., some scores overlapped).

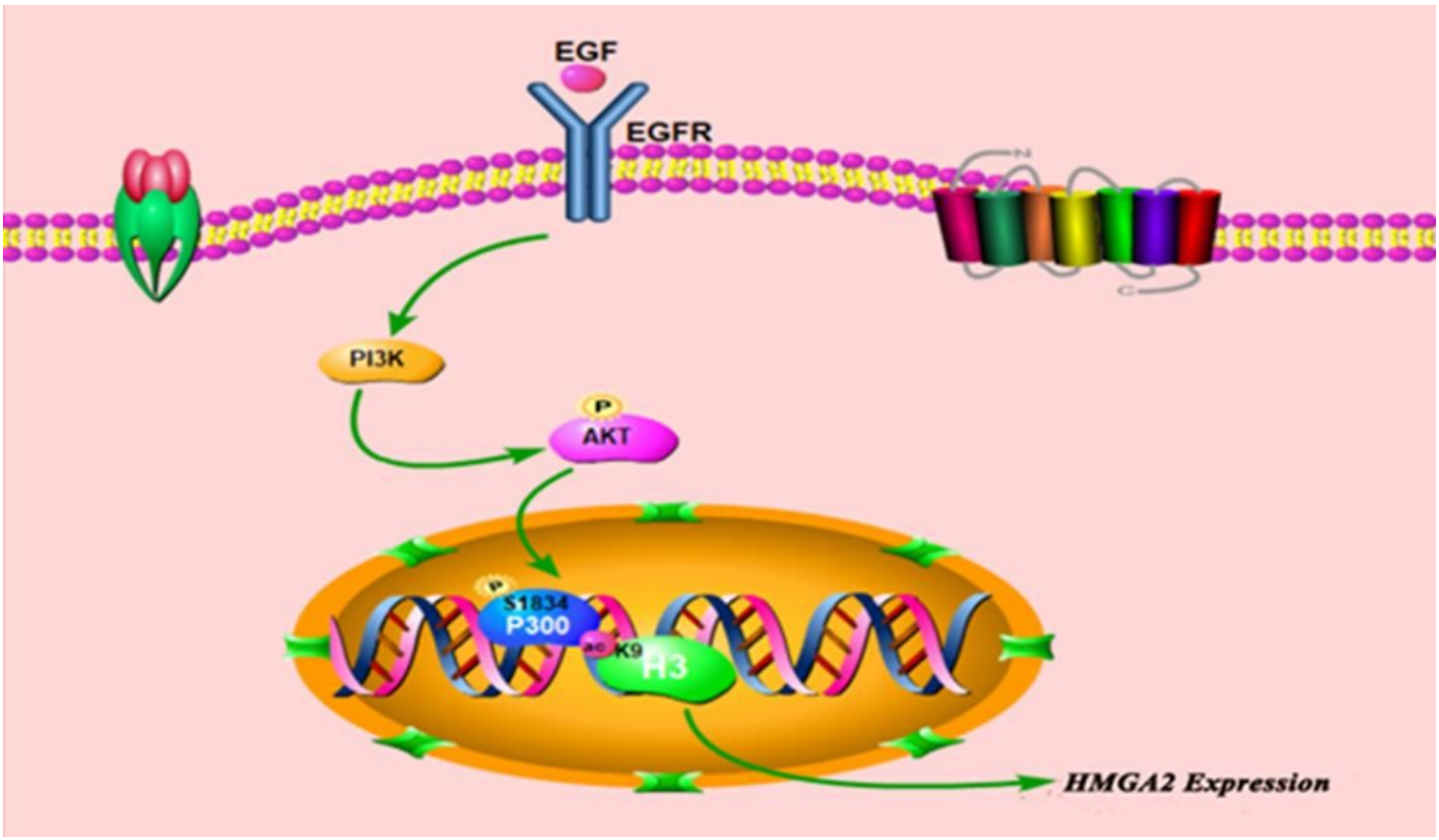

Figure 6

Schematic representation of EGF-mediated HMGA2 transcription in hepatocellular carcinoma cells. EGF can induce p300 phosphorylation at Ser1834 site through activation of PI3K/AKT signaling pathway in hepatocellular carcinoma, p300 subsequently catalyzes histone H3 acetylation at K9 residues, leading to HMGA2 transcription in HCC.