Oxymatrine blocked mother-to-child transmission of hepatitis B virus by modulating autophagy in trophoblast cells

Keywords
Autophagy, Oxymatrine, Hepatitis B virus, Mother-to-child transmission, Trophoblast cell

Abstract

Introduction
Mother-to-child transmission is a significant pathway for chronic carriers of the hepatitis B virus (HBV) in China. In this study, we aimed to investigate the role and mechanism of Oxymatrine (OMT) in preventing mother-to-child transmission of HBV.

Material and methods
To simulate MTCT, we utilized the HBV-infected human trophoblast cell line HTR-8/SVneo, which serves as a relevant model for studying HBV transmission at the maternal-fetal interface. The replication capacity of HBV in these cells was quantified using enzyme-linked immunosorbent assay (ELISA) and real-time fluorescence polymerase chain reaction (PCR). The expression levels of key autophagy markers were assessed using Western blotting, providing insights into the autophagy-related mechanisms potentially involved. Additionally, the Cell Counting Kit-8 (CCK-8) assay was employed to measure the proliferation of trophoblast cells under different treatment conditions.

Results
We found that OMT inhibited HBV DNA replication in HBV-infected trophoblast cells. Additionally, OMT suppressed the proliferation and autophagy in HBV-infected trophoblast cells. This suggested that OMT might effectively block mother-to-child transmission of HBV. Mechanistically, OMT appears to prevent mother-to-child transmission of HBV by inhibiting the EGFR/Akt pathway.

Conclusions
OMT inhibited HBV transmission by regulating the EGFR/Akt pathway, and this study may provide new ideas and methods for the prevention of mother-to-child transmission of HBV infection during pregnancy.

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Keywords: Oxymatrine; Hepatitis B virus; Trophoblast cell; Autophagy; Mother-to-child transmission

Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus that causes persistent liver infections in humans [1, 2]. Chronic HBV infection is a significant risk factor for hepatocellular carcinoma, accounting for 50-80% of liver cancer cases worldwide [2].

The primary mode of transmission for chronic HBV infection is mother-to-child transmission [3]. Although only 5-15% of HBV-positive infants are infected through intrauterine transmission, this route remains one of the most common pathways for HBV transmission [4]. The relatively low rate of mother-to-child transmission, ranging from 5-15%, can be attributed to the greater resistance of placental trophoblast cells to viral infection compared to non-trophoblast cells [5]. Nevertheless, human trophoblasts can still be infected by HBV, leading to the aforementioned infant infection rate. Therefore, it is crucial to investigate the mechanisms underlying the resistance of placental trophoblasts to HBV.

Autophagy is the process by which cells degrade and recycle proteins and organelles to maintain intracellular homeostasis [6]. It also plays a role in the immune response to various pathogens. Research has demonstrated that autophagy can facilitate the replication of a range of viruses, including coronaviruses, flaviviruses, and the hepatitis C virus (HCV) [7-9]. Additionally, autophagy in placental cells may significantly contribute to the vertical transmission of HBV. Previous studies indicated that autophagy was elevated in maternal placentas infected with HBV, and the level of autophagy was notably upregulated with increasing HBV viral load [10]. Consequently, the *in vitro* inhibition of trophoblast autophagy markedly enhances antiviral activity, which is crucial in preventing HBV transmission from mother to child. Furthermore, it has been observed that HBV-infected trophoblast cells exhibited reduced apoptotic capacity and increased migratory ability [11]. In summary, inhibiting autophagy and promoting apoptosis in HBV-infected trophoblast cells may be advantageous in obstructing mother-to-child transmission of HBV. Nonetheless, effective therapeutic options for interrupting mother-to-child transmission of HBV need to be explored.

In China, many traditional Chinese medicines and their derivatives are widely utilized in antiviral therapy [12-14]. Oxymatrine (OMT), an alkaloid extracted from the *herb Sophora alopecuraides L.* [15], has demonstrated significant inhibition of HBV replication in HBV-transfected cell lines, HBV-transgenic mouse models, and patients with chronic hepatitis B [16-18]. However, the role and mechanism of OMT in mother-to-child transmission remain unclear and require further investigation.

In this study, we found that OMT inhibited proliferation and autophagy in HBV-infected trophoblast cells by suppressing the activation of the EGFR/Akt pathway. This mechanism may contribute to blocking mother-to-child transmission of HBV.

Materials and methods

Cell culture

The trophoblast cell line HTR-8/SVneo was provided by the American Typical Culture Collection (ATCC, USA) and cultured in RPMI-1640 medium (Hyclone, USA) supplemented with 10% FBS, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37℃ with 5% CO_2.

Hepatitis B virus infection

Referring to the previous method [19], serum samples were collected from HBV carriers with high levels of HBV DNA (>1.0×10^8 copies/ml), and bacteria were filtered out using a 0.22 μm filtration device (Millipore, China). HTR-8/SVneo cells were incubated with 30% HBV positive serum (HBV titer: 1×10^7) for 72 hours at 37℃.

Treatment of HTR-8/SVneo cells with OMT

After 72 hours of co-culture of HTR-8/SVne with HBV positive serum, the medium was discarded and fresh medium containing 100 mg/l, 200 mg/l and 400 mg/l OMT was added, and the cells were collected for assaying at 48 hours after treatment. For the Isoprocurcumenol administration rescue group, we treated the cells with medium containing 1 μM Isoprocurcumenol for 48 hours.

Enzyme-linked immunosorbent assay (ELISA)

Forty-eight hours after OMT treatment, cell supernatants were collected and assayed for both HBsAg and HBeAg using an ELISA kit (Shanghai Zhongmei Biotech Co., Ltd., China) according to the manufacturer's instructions. The samples and enzyme conjugates were added to 96-well ELISA plates at 100 μL/well, respectively, and incubated at 37°C for 1 h. The plates were washed 5 times with plate wash solution from the kit and patted dry. Add Color Developing Solution A and B to each well and incubate at 37℃ for 15 minutes, avoiding light to help color development. Add the termination solution, shake gently to mix, and read the absorbance at 450 nm within 30 minutes.

Quantification of HBV DNA

HBV DNA in the supernatant was purified using the QIAamp DNA Mini Kit (QIAGEN Inc., USA) according to the manufacturer's instructions, followed by real-time fluorescence PCR using the primers RCP1/RCP2 and the Taqman ® Minor Groove Binding Complex (MGB) Probe DRC, which was used to target the HBV genome's S open reading frame (ORF). RCP1/RCP2 sequences are shown below: RCP1, 5'TCCTCTTCATCCTGCTGCTATG 3', RCP2, 5'CGTGCTGGTAGTTGATGTTCCT 3'.

Cell Counting Kit 8 (CCK-8)

Cell viability was determined by CCK-8. HTR-8/SVneo cells (1×10^4 cells /well) were seeded into 96-well plates. 10 μL of CCK-8 solution (Beyotime, China) was added to the medium. After 4 hours of incubation, the sample absorbance (450 nm) was measured using a spectrophotometer (Multiscan MK3, Thermo Fisher Scientific, USA) at various times.

Western blotting (WB)

Total protein was extracted from HTR-8/SVneo cells using RIPA lysis buffer (Beyotime, China). The proteins were then separated by 10% SDS electrophoresis and transferred to a PVDF membrane. The membranes were then closed with 5% skimmed milk for one hour at room temperature and then incubated overnight at 4°C with primary antibodies. After washing the membranes, the membranes are incubated with HRP-labeled IgG secondary antibody for 1 hour at 37°C. Finally, the membranes were analyzed using the ECL protein blotting test (Beyotime, China). Table 1 shows the antibodies used in the study.

Table 1. Antibody Information.

	Gene name	Catalog Number	Manufacturer	Dilution ratios
WB	P62	ab109012	abcam	1:5000
	LC3	ab108327	abcam	1:2000
	p-EGFR	ab40815	abcam	1:500
	EGFR	ab52894	abcam	1:500
	p-AKT	4060	Cell Signaling Technology, Inc.	1:400

AKT	4691T	Cell Signaling Technology, Inc.	1:400
β-actin	ab8226	abcam	1:3000
Goat Anti-Rabbit	S0001	Affinity	1:3000

Statistical analysis

Statistical tests were performed using SPSS 22.0 statistical software. Data were expressed as mean ± standard deviation (SD). Unpaired Student's t-test was used for comparisons between two groups, and one-way ANOVA with post hoc Bonferroni correction was used for multiple comparisons. Image data were collected and processed using Graphpad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

Results

OMT inhibited the replicative ability of HBV in trophoblast cells

HBV patient serum has the capacity to infect trophoblast cells *in vitro*, making it a valuable tool for establishing an experimental model that simulates the interaction between HBV and the trophoblast barrier. Subsequently, HBV-infected HTR-8/SVneo cells were treated with OMT to investigate the possible role of OMT in the replication of HBV. We first examined the levels of HBsAg and HBeAg in the supernatants of HTR-8/SVneo cells. The results indicated that HBV increased the levels of HBsAg and HBeAg compared to untreated cells, while treatment with OMT reduced HBsAg and HBeAg levels in a dose-dependent manner (Figure 1A and 1B). In addition, OMT was found to inhibit the replication capacity of HBV in a dose-dependent manner as determined by measuring HBV DNA load (Figure 1C). The above results suggested that OMT reduced the replication ability of the HBV virus in a dose-dependent manner in trophoblast cells.

OMT regulated autophagy and proliferation in HBV-infected trophoblast cells

The biological effects of HBV and OMT on HTR-8/SVneo cells have been investigated in this study. Cell viability was assessed using the CCK-8 assay, and Figure 2A demonstrates that HBV significantly induced HTR-8/SVneo cell viability ($P < 0.05$), whereas OMT notably reduced cell viability following HBV infection (Figure 2A, $P < 0.05$). WB analysis revealed that HBV significantly increased the expression of

autophagy proteins LC3 II and ATG5 while decreasing the expression of P62. In contrast, OMT reversed these protein level changes (Figure 2B and 2C). These results suggest that HBV induces autophagy, whereas OMT inhibits it. In summary, OMT inhibited autophagy and cell viability in HBV-infected HTR-8/SVneo cells.

OMT repressed the EGFR/Akt pathway

Previous studies have demonstrated that OMT can modulate the activation of the EGFR and AKT pathways. In this study, we further investigated the molecular mechanisms by which OMT regulates these pathways in HBV-infected HTR-8/SVneo cells. Notably, the results indicated that HBV increased the levels of EGFR and AKT phosphorylation compared to the control group (Figure 3). However, following OMT treatment, the phosphorylation levels of both EGFR and AKT were significantly reduced in HBV-infected HTR-8/SVneo cells (Figure 3, $P < 0.05$). In conclusion, these findings suggested that OMT inhibited the EGFR/AKT pathway in HBV-infected trophoblast cells.

Activation of the EGFR/Akt pathway reversed the protective effect of OMT in HBV-infected trophoblast cells

To investigate whether OMT exerts antiviral and cytoprotective functions by regulating the EGFR/AKT pathway, we treated cells with Isoprocurcumenol, an agonist of the EGFR/AKT pathway. Our findings indicated that OMT reduced the phosphorylation of both EGFR and AKT in HBV-infected HTR-8/SVne cells, while Isoprocurcumenol restored the phosphorylation levels diminished by OMT (Figure 4A-4C). We also examined the replication capacity of HBV and discovered that OMT decreased the levels of HBsAg, and HBeAg. Conversely, Isoprocurcumenol reversed these changes, suggesting that it enhanced the replication capacity of HBV inhibited by OMT (Figure 4D and 4E). Furthermore, OMT reduced the viability of HBV-infected HTR-8/SVne cells, whereas Isoprocurcumenol restored cell viability (Figure 4F). The WB analysis of autophagy markers demonstrated that Isoprocurcumenol re-promoted the expression of LC3 II and ATG5 while decreasing the expression of P62 (Figure 4G and 4H). Taken together, the results indicated that the antiviral and protective effects of OMT in HBV-infected trophoblast cells were mediated through the modulation of the

EGFR/AKT pathway.

Discussion

This study demonstrated that OMT inhibits HBV replication in trophoblast cells by modulating autophagy by suppressing the EGFR/Akt signaling pathway. This finding suggests a novel mechanism by which OMT may help prevent mother-to-child transmission of HBV at the placental level. Given that intrauterine transmission remains difficult to avoid in pregnant women with high viral loads, our results offer a potential therapeutic strategy that targets viral transmission at its earliest point of fetal contact. Below, we discuss how our findings integrate with existing literature and propose directions for future research.

The placenta serves as a physical barrier between the mother and fetus and is closely associated with the mother-to-child transmission of various viruses, including HBV [20]. Mother-to-child transmission of most HBV has been effectively controlled due to the widespread use of hepatitis B immunoglobulin and hepatitis B vaccine [3, 21]. However, these treatments do not appear to prevent mother-to-child transmission in pregnant women with high HBV loads. Therefore, blocking intrauterine transmission of HBV remains an unresolved challenge. The trophoblast, the outermost layer of the placenta, represents the first barrier HBV must overcome to pass through the placenta, as it comes into direct contact with maternal blood during pregnancy [21, 22]. Research has shown that HBV can reach the placenta via maternal blood, infect placental trophoblast cells, survive, replicate within these cells, and produce the functional protein HBxAg [23]. Therefore, investigating the role of HBV-infected placental trophoblasts is crucial for elucidating the mechanisms of intrauterine HBV transmission and may provide insights for developing novel therapeutic strategies. This study employed HBV-infected trophoblasts as the primary model to examine intrauterine HBV transmission, with a specific focus on evaluating the inhibitory effects of OMT on HBV replication.

Hong Cui demonstrated that HBV inhibited apoptosis and enhanced cell proliferation in HTR-8/SVneo cells [11]. Bai et al. found that the apoptotic index of HBV-infected, highly replicating cells was lower than that of uninfected cells [23].

Interestingly, the high proliferation of trophoblast cells favors embryo implantation [24, 25]. If the proliferation of HBV-infected trophoblast cells is increased, there is a greater likelihood of mother-to-child transmission of HBV. Consistent with previous findings, in the current study, we found that HBV induced the proliferation of trophoblast cells. These results demonstrated that HBV promoted proliferation to increase the risk of mother-to-child transmission of the virus. The activation of autophagy in placental cells plays a crucial role in the mechanism of HBV mother-to-child transmission [26]. Recent studies have shown that HBV infection can induce autophagy in a manner dependent on HBS and HBX proteins [27]. Activation of autophagy promotes HBV DNA replication, viral particle envelopes, and HBV release from host cells [28, 29]. Furthermore, autophagy in placental cells may significantly enhance the vertical transmission of HBV [30]. Notably, elevated autophagic markers have been observed in placentas from HBV-infected mothers, with all autophagy-related genes demonstrating significant upregulation in correlation with increasing HBV viral load [10]. Consistent with previous findings, in the current study, we indicated that HBV increased the autophagic capacity of trophoblast cells, which aligned with previously reported results. These results demonstrated that HBV promoted mother-to-child transmission by mediating autophagy.

In recent years, herbal extracts have gained popularity in antiviral therapy [12-14]. OMT is an alkaloid derived from Medicago sativa [31]. Numerous studies have demonstrated that OMT significantly inhibited HBV DNA replication in cellular models and mice [32, 33]. Furthermore, OMT has been utilized in clinical practice for treating patients with HBV-related liver injury, showing high safety and applicability [18, 34, 35]. However, there is limited research on the potential of OMT to prevent mother-to-child transmission of HBV. This study evaluated the efficacy of OMT in HBV-infected trophoblast cells. The results indicated that OMT inhibited HBV replication in these cells, consistent with previous findings that OMT suppresses HBV DNA replication [32, 33]. Additionally, OMT reduced the proliferation and autophagy of HBV-infected trophoblast cells. Therefore, OMT has the potential to block mother-to-child transmission of HBV.

In addition, this study further demonstrated that OMT inhibited the EGFR/Akt signaling pathway. Previous studies have established the critical role of EGFR/Akt signaling in regulating cell proliferation, cytokine production, and hormone secretion [36, 37]. Wang et al. confirmed that HBV reduced trophoblast apoptosis and enhanced placental hormone secretion through EGFR/Akt pathway activation [38]. Consistently, HBV-infected human placental tissues and trophoblasts exhibited significant upregulation of p-EGFR protein, with HBV suppressing trophoblast apoptosis via EGFR/Akt activation [39] - findings that align with our current results and suggest HBV-mediated EGFR/Akt pathway stimulation. Notably, OMT specifically targeted p-EGFR to suppress downstream Akt phosphorylation [40-42], which corroborates our observed mechanism of EGFR/Akt inhibition by OMT. Collectively, these results indicate that OMT blocks mother-to-child HBV transmission by suppressing EGFR/Akt pathway activity in HBV-infected trophoblasts.

Despite this study's innovative proposal regarding the role of OMT in preventing mother-to-child transmission of HBV, several limitations should be acknowledged. Firstly, the study lacks results from clinical trials of OMT for validation purposes. Future research should involve recruiting HBV-infected pregnant women to undergo OMT to assess its effectiveness in controlling HBV. Secondly, further investigation is needed to explore additional molecular mechanisms through which OMT inhibits HBV replication. Third, this study did not include experiments with autophagy inhibitors to confirm the role of autophagy modulation in OMT's action. This represents a limitation that should be addressed in future studies to further substantiate the findings and refine our understanding of the underlying mechanisms. Lastly, while this study used WB to quantify autophagy markers, future studies incorporating immunofluorescence microscopy are needed to provide spatial confirmation of autophagic changes at the cellular level.

In summary, our study demonstrated that OMT inhibited cell autophagy and proliferation in HBV-infected trophoblast cells by suppressing the activation of the EGFR/Akt pathway. This mechanism may contribute to blocking mother-to-child transmission of HBV.

Funding

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

Not applicable.

Conflict of interest statement

The authors have no competing interests to declare.

References

1. Liu, Z., et al., *Exosomes in HBV infection.* Clin Chim Acta, 2023. **538**: p. 65-69.
2. Chen, Y. and Z. Tian, *HBV-Induced Immune Imbalance in the Development of HCC.* Front Immunol, 2019. **10**: p. 2048.
3. Jing, W., J. Liu, and M. Liu, *Eliminating mother-to-child transmission of HBV: progress and challenges in China.* Front Med, 2020. **14**(1): p. 21-29.
4. Chen, Y., et al., *Role of maternal viremia and placental infection in hepatitis B virus intrauterine transmission.* Microbes Infect, 2013. **15**(5): p. 409-15.
5. Delorme-Axford, E., et al., *Human placental trophoblasts confer viral resistance to recipient cells.* Proc Natl Acad Sci U S A, 2013. **110**(29): p. 12048-53.
6. Liu, S., et al., *Autophagy: Regulator of cell death.* Cell Death Dis, 2023. **14**(10): p. 648.
7. Chiramel, A.I. and S.M. Best, *Role of autophagy in Zika virus infection and pathogenesis.* Virus Res, 2018. **254**: p. 34-40.
8. Miller, K., et al., *Coronavirus interactions with the cellular autophagy machinery.* Autophagy, 2020. **16**(12): p. 2131-2139.
9. Chan, S.T. and J.J. Ou, *Hepatitis C Virus-Induced Autophagy and Host Innate Immune Response.* Viruses, 2017. **9**(8).
10. Gao, H., et al., *The Role of Autophagy in the Mother-to-Child Transmission of Pregnant Women With a High Level of HBV DNA.* Front Cell Infect Microbiol, 2022. **12**: p. 850747.
11. Cui, H., et al., *Hepatitis B virus X protein modifies invasion, proliferation and the inflammatory response in an HTR-8/SVneo cell model.* Oncol Rep, 2015. **34**(4): p. 2090-8.
12. Dai, Y.J., et al., *Recent advances of traditional Chinese medicine on the prevention and treatment of COVID-19.* Chin J Nat Med, 2020. **18**(12): p. 881-

889.
13. Li, T. and T. Peng, *Traditional Chinese herbal medicine as a source of molecules with antiviral activity.* Antiviral Res, 2013. **97**(1): p. 1-9.
14. Ren, W., et al., *Research progress of traditional Chinese medicine against COVID-19.* Biomed Pharmacother, 2021. **137**: p. 111310.
15. Lan, X., et al., *Oxymatrine exerts organ- and tissue-protective effects by regulating inflammation, oxidative stress, apoptosis, and fibrosis: From bench to bedside.* Pharmacol Res, 2020. **151**: p. 104541.
16. Sang, X., et al., *T cell--associated immunoregulation and antiviral effect of oxymatrine in hydrodynamic injection HBV mouse model.* Acta Pharm Sin B, 2017. **7**(3): p. 311-318.
17. Xu, W.S., et al., *Effect of oxymatrine on the replication cycle of hepatitis B virus in vitro.* World J Gastroenterol, 2010. **16**(16): p. 2028-37.
18. Song, W.J., et al., *Oral oxymatrine preparation for chronic hepatitis B: A systematic review of randomized controlled trials.* Chin J Integr Med, 2016. **22**(2): p. 141-9.
19. Cui, H., J. Chen, and Q. Na, *Effect of hepatitis B virus infection on trophoblast cell line (HTR-8/SVneo) and choriocarcinoma cell line (JEG3) is linked to CD133-2 (AC141) expression.* Am J Transl Res, 2016. **8**(7): p. 3235-40.
20. Cheung, K.W., M.T.Y. Seto, and T.T. Lao, *Prevention of perinatal hepatitis B virus transmission.* Arch Gynecol Obstet, 2019. **300**(2): p. 251-259.
21. Ma, L., et al., *Mother-to-child transmission of HBV: review of current clinical management and prevention strategies.* Rev Med Virol, 2014. **24**(6): p. 396-406.
22. Mavilia, M.G. and G.Y. Wu, *Mechanisms and Prevention of Vertical Transmission in Chronic Viral Hepatitis.* J Clin Transl Hepatol, 2017. **5**(2): p. 119-129.
23. Bai, G., et al., *The study on the role of hepatitis B virus X protein and apoptosis in HBV intrauterine infection.* Arch Gynecol Obstet, 2012. **285**(4): p. 943-9.
24. Abbas, Y., et al., *Investigation of human trophoblast invasion in vitro.* Hum Reprod Update, 2020. **26**(4): p. 501-513.
25. Lai, T.H., H.T. Chen, and W.B. Wu, *Trophoblast coculture induces intercellular adhesion molecule-1 expression in uterine endometrial epithelial cells through TNF-α production: Implication of role of FSH and ICAM-1 during embryo implantation.* J Reprod Immunol, 2022. **152**: p. 103650.
26. Jackson, W.T., *Autophagy as a broad antiviral at the placental interface.* Autophagy, 2013. **9**(12): p. 1905-7.
27. Liu, D.X., et al., *Exosomes derived from HBV-associated liver cancer promote chemoresistance by upregulating chaperone-mediated autophagy.* Oncol Lett, 2019. **17**(1): p. 323-331.
28. He, Q., et al., *Dexamethasone Stimulates Hepatitis B Virus (HBV) Replication Through Autophagy.* Med Sci Monit, 2018. **24**: p. 4617-4624.
29. Wu, S.Y., S.H. Lan, and H.S. Liu, *Autophagy and microRNA in hepatitis B virus-*

related hepatocellular carcinoma. World J Gastroenterol, 2016. **22**(1): p. 176-87.

30. Wang, D.D., et al., *Relationship between Maternal PBMC HBV cccDNA and HBV Serological Markers and its Effect on HBV Intrauterine Transmission.* Biomed Environ Sci, 2019. **32**(5): p. 315-323.
31. Huan, D.Q., N.Q. Hop, and N.T. Son, *Oxymatrine: A current overview of its health benefits.* Fitoterapia, 2023. **168**: p. 105565.
32. Lin, M., et al., *Inhibition of the replication of hepatitis B virus in vitro by oxymatrine.* J Int Med Res, 2009. **37**(5): p. 1411-9.
33. Lu, L.G., et al., *Inhibitory effect of oxymatrine on serum hepatitis B virus DNA in HBV transgenic mice.* World J Gastroenterol, 2004. **10**(8): p. 1176-9.
34. Jiang, X., et al., *Oral oxymatrine for hepatitis B cirrhosis: A systematic review protocol.* Medicine (Baltimore), 2018. **97**(49): p. e13482.
35. Lu, L.G., et al., *Oxymatrine therapy for chronic hepatitis B: a randomized double-blind and placebo-controlled multi-center trial.* World J Gastroenterol, 2003. **9**(11): p. 2480-3.
36. Cui, X., et al., *Blockage of EGFR/AKT and mevalonate pathways synergize the antitumor effect of temozolomide by reprogramming energy metabolism in glioblastoma.* Cancer Commun (Lond), 2023. **43**(12): p. 1326-1353.
37. Jing, X., et al., *IGF2BP3-EGFR-AKT axis promotes breast cancer MDA-MB-231 cell growth.* Biochim Biophys Acta Mol Cell Res, 2023. **1870**(8): p. 119542.
38. Wang, W., et al., *HBxAg suppresses cell apoptosis and promotes the secretion of placental hormones in human placental trophoblasts via activation of the EGFR/Akt pathway.* Cell Biol Int, 2018. **42**(2): p. 237-247.
39. Lin, Y., et al., *HBxAg promotes HBV replication and EGFR activation in human placental trophoblasts.* Exp Ther Med, 2021. **22**(5): p. 1211.
40. Guo, B., et al., *Oxymatrine targets EGFR(p-Tyr845) and inhibits EGFR-related signaling pathways to suppress the proliferation and invasion of gastric cancer cells.* Cancer Chemother Pharmacol, 2015. **75**(2): p. 353-63.
41. Halim, C.E., et al., *Anti-cancer effects of oxymatrine are mediated through multiple molecular mechanism(s) in tumor models.* Pharmacol Res, 2019. **147**: p. 104327.
42. Dai, Z., et al., *Oxymatrine induces cell cycle arrest and apoptosis and suppresses the invasion of human glioblastoma cells through the EGFR/PI3K/Akt/mTOR signaling pathway and STAT3.* Oncol Rep, 2018. **40**(2): p. 867-876.

Figure legends

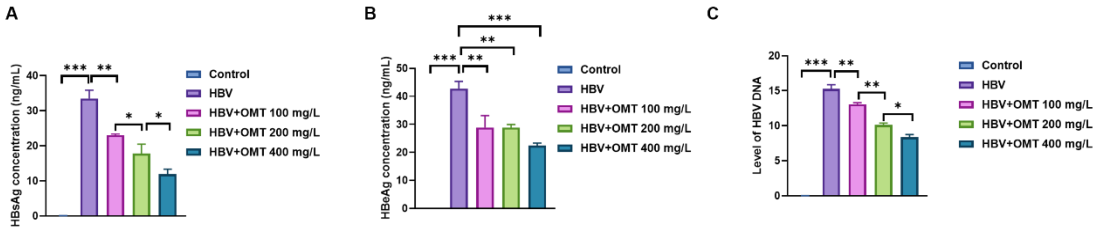


Figure1. OMT inhibited the replicative ability of HBV in trophoblast cells.

HTR-8/SVneo cells were infected with HBV or treated with OMT (100mg/ml, 200mg/ml, and 400mg/ml). (A) HBsAg in cell supernatant were detected by ELISA. (B) HBeAg in cell supernatant were detected by ELISA. (C) HBV DNA load in cell were detected by real-time fluorescence PCR. Data are presented as mean ± SD. *P<0.05, **P<0.01, ***P<0.001.

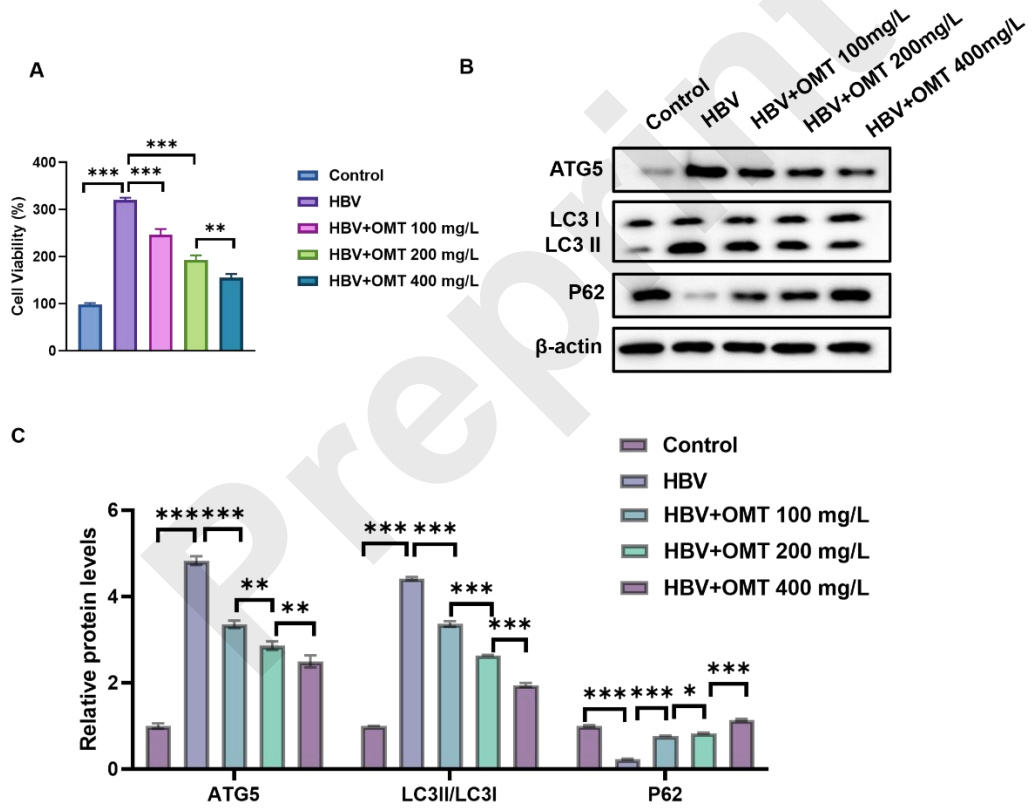


Figure2. OMT regulated autophagy and apoptosis in HBV-infected trophoblast cells.

HTR-8/SVneo cells were infected with HBV or treated with OMT (100mg/ml, 200mg/ml, and 400mg/ml). (A) Cell viability was detected by CCK-8. (B-C) The protein expression of LC3 I, LC3 II and ATG5 was detected by WB. Data are presented as mean ± SD. *P<0.05, **P<0.01, ***P<0.001.

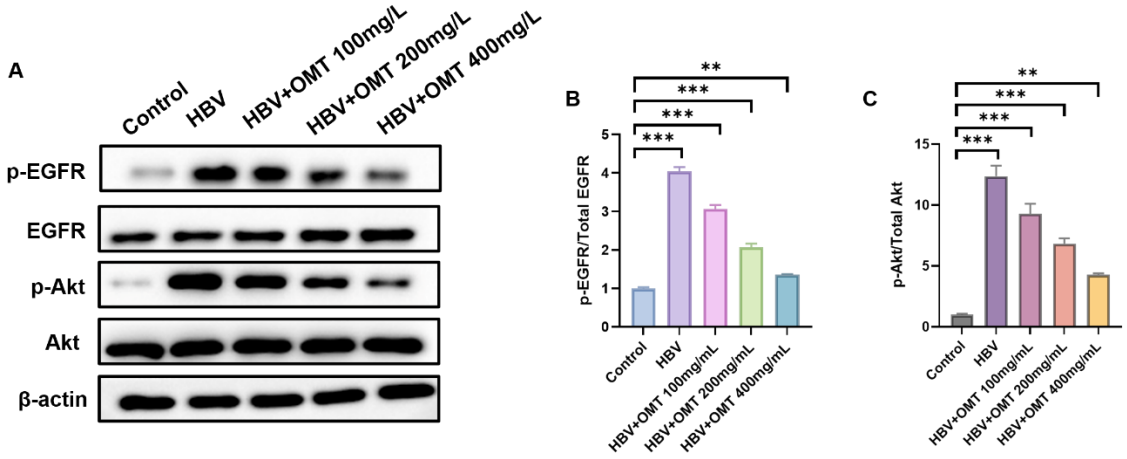
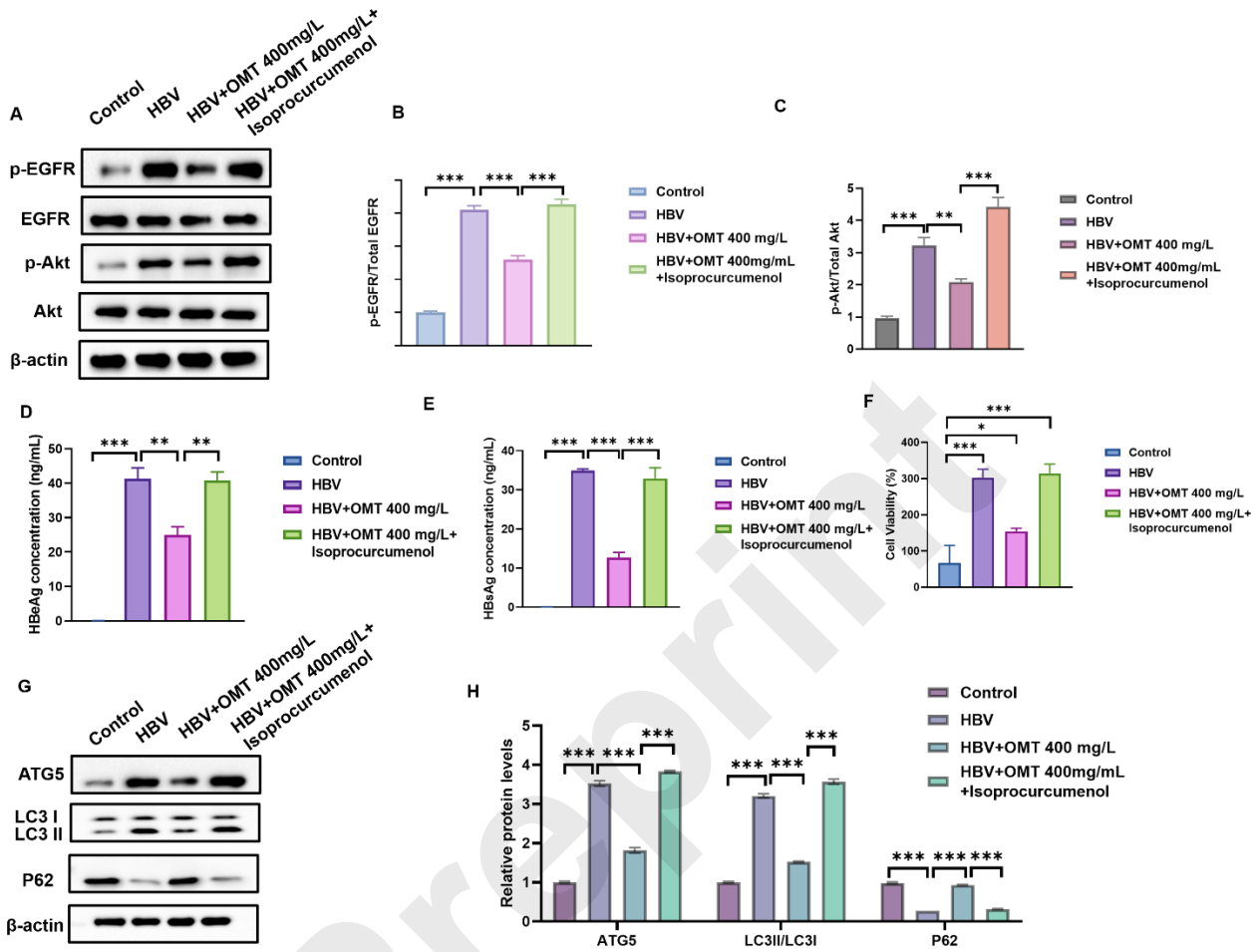


Figure3. OMT repressed the EGFR/Akt pathway.

HTR-8/SVneo cells were infected with HBV or treated with OMT (100 mg/ml, 200 mg/ml, and 400 mg/ml). (A-C) The protein expression of p-EGFR, EGFR, p-AKT and AKT was detected by WB. Data are presented as mean ± SD. *P<0.05, **P<0.01,

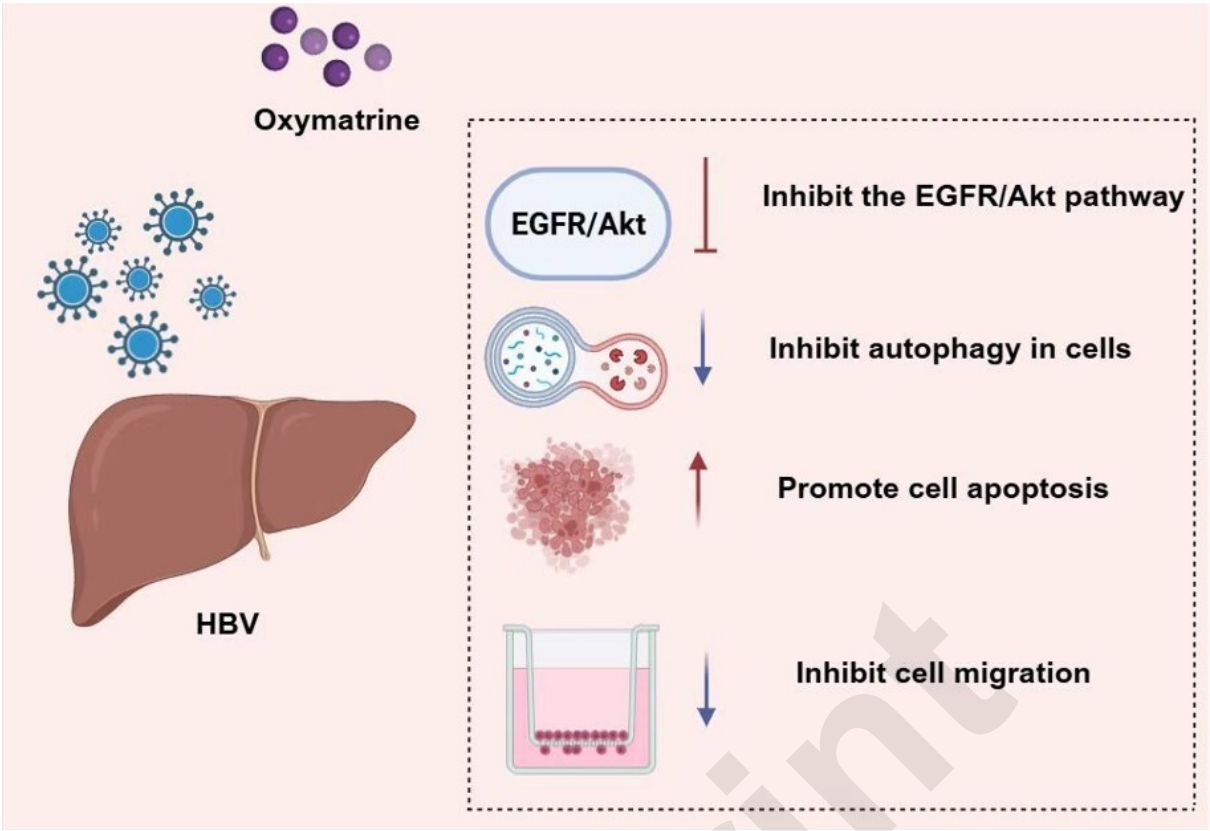
***P<0.001.

Figure4. Activation of the EGFR/Akt pathway reversed the protective effect



of OMT in HBV-infected trophoblast cells.

HTR-8/SVneo cells were infected with HBV or treated with OMT (400mg/ml) and Isoprocurcumenol (1 μM). (A-C) The protein expression of p-EGFR, EGFR, p-AKT and AKT was detected by WB. (D-E) HBsAg and HBeAg in cell supernatant were detected by ELISA. (F) Cell viability was detected by CCK-8. (G-H) The protein expression of LC3 I, LC3 II and ATG5 was detected by WB. Data are presented as mean ± SD. *P<0.05, **P<0.01, ***P<0.001.


Oxymatrine
HBV
EGFR/Akt
Inhibit the EGFR/Akt pathway
Inhibit autophagy in cells
Promote cell apoptosis
Inhibit cell migration