Discover Oncology

Research

Association of metformin with risk of digestive tract cancers from a drug target mendelian randomization and cell experiments

Yifei Wang[1,2] · Xirong Cao[1,2] · Jie Ren[1,2] · Rui Chen[1,2] · Xing Zhang[1,2] · Chang Liu[1,2] · Yifan Jia[2,3] · Ting Lin[2,4]

Received: 10 March 2025 / Accepted: 16 May 2025
Published online: 23 May 2025

Abstract

Background Digestive tract cancers account for a significant proportion of the global cancer burden, and their prevention and treatment pose a worldwide challenge. Metformin, as a first-line treatment for diabetes, offers advantages such as high safety and affordability. Previous research has suggested that the use of metformin may reduce the risk of cancers, but there is still a lack of strong evidence regarding its causal relationship with digestive tract cancers.

Methods We employed Mendelian randomization (MR) analysis to investigate the causal relationships between metformin use and various digestive tract cancers. We selected single nucleotide polymorphisms (SNPs) related to the Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK), which is associated with the action of metformin, as instrumental variables. The inverse variance-weighted method (IVW) was the most important method. Cochran's Q was used to detect heterogeneity, and the MR-PRESSO test and MR-Egger regression were used to detect horizontal pleiotropy. Subsequently, we verified the toxicity and proliferation inhibition of metformin on Huh 7 and PLC in hepatocellular carcinoma cells.

Results IVW results showed that metformin use reduced the risk of liver and bile duct cancers (OR = 0.994, 95% CI 0.990–0.999; P = 0.008), but there were no causal relationships with other digestive tract cancers. Our cell experiments have confirmed this point.

Conclusion Metformin may be used for the prevention or treatment of liver and bile duct cancers.

Keywords Metformin · Digestive tract cancer · Mendelian randomization · AMPK

1 Introduction

Digestive tract cancers include esophageal cancer, liver and bile duct cancers, colorectal cancer, pancreatic cancer, and others. They are a significant cause of cancer-related deaths globally [1]. According to the latest epidemiological statistics related to cancers, in 2023, among new cases of invasive cancer in the United States, digestive tract cancers rank second only to reproductive system cancers. It is estimated that in 2023, the number of deaths due to digestive tract cancers will

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-02729-3.

✉ Yifan Jia, jiayifanivan@foxmail.com; ✉ Ting Lin, linda_ting@xjtufh.edu.cn | [1]Department of Hepatobiliary Surgery and Liver Transplantation, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China. [2]Key Laboratory of Surgical Critical Care and Life Support (Xi'an Jiaotong University), Ministry of Education, Xi'an 710061, Shaanxi, China. [3]Department of Vascular Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China. [4]Department of Surgical Critical Care, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China.

be significantly higher than all other invasive cancers, approximately 172,010 people [2]. Effective treatments for digestive tract cancers are currently lacking.

As is well known, metformin can inhibit hepatic gluconeogenesis by activating Adenosine 5 '-monophosphate (AMP)-activated protein kinase (AMPK) [3] and enhance insulin-mediated glucose uptake in skeletal muscle cells. It is currently the most widely used anti-diabetic medication in clinical practice [4]. In recent years, an increasing body of research supports the association between metformin and a reduced risk of cancer incidence and mortality [3]. Metformin treatment can reduce the risk of biliary tract cancer (BTC) by 60% in diabetes patients [5, 6]. Metformin can also inhibit the apoptosis, invasion, and migration of rectal cancer cells by suppressing TGFBR2 signaling [7]. A prospective cohort study conducted in Sweden showed that metformin can lower the risk of developing esophageal squamous cell carcinoma (ESCC) [8]. Despite mounting evidence suggesting that metformin can reduce the risk of cancer incidence and mortality [9–11], there are also contradictory research findings [12]. For instance, in a Swedish nationwide cohort study involving 50,000 individuals, the authors evaluated the relationship between metformin and the risk of BTC and its subtypes and found no significant association between the two [13]. Therefore, the role of metformin on digestive tract cancers remains controversial. The existence of drug indications and ethical concerns limits the conduct of randomized controlled trials (RCTs) for metformin intervention in digestive tract cancers.

Mendelian randomization (MR) is an analytical method that uses genetic variation as an instrumental variable. Because the allele genes associated with exposure are randomly allocated, and the study results are not influenced by reverse causation, it is often used to assess causal relationships between modifiable exposures or risk factors and clinical events [14–17]. Mendelian randomization employs genetic variation as a substitute for exposure factors in causal analysis, which is not constrained by ethical factors and is easier to conduct than RCTs. Therefore, this study aims to explore the effects of metformin on digestive tract cancers through Mendelian randomization.

2 Methods

2.1 Study design and participants

This is a two-sample MR study, and Fig. 1 depicts the design flowchart of this study. The aim of our study was to investigate the causal effect of metformin on digestive tract cancers, including liver and bile duct cancers, esophageal cancer, pancreatic cancer, colorectal cancer, rectal cancer, and oral cancer. We chose the activation of AMPK as a genetic instrument representing the targeted effects of metformin [14].

Figure 2 illustrates the principles of MR. MR is based on three assumptions: (A) single nucleotide polymorphisms (SNPs) are strongly associated with glycated hemoglobin (HbA1c); (B) SNPs are independent of known confounders; (C) SNPs only affect digestive tract cancer through HbA1c [18].

2.2 Genetic instrument selection

We employed the same approach as previous studies [17], initially searching for SNPs within 1 mega base pairs upstream and downstream of genes encoding AMPK subunits. We specifically chose SNPs that exhibited low linkage disequilibrium ($r^2 < 0.3$) with HbA1c to minimize population stratification. This selection was limited to individuals of European descent. Subsequently, we validated the association between these selected SNPs and HbA1c in the UK Biobank, retaining only those variants that reached statistical significance in the UK Biobank ($p \leq 0.05$).

2.3 Study outcome

We used data from the UK Biobank, PanScan1, and MRC-IEU databases. liver and bile duct cancers (n = 350,372,016), esophageal cancer (n = 740,372,016), and oral cancer (n = 357,372,016) all came from the UK Biobank. Colorectal cancer (n = 1,494,461,439) and rectal cancer (n = 1,085,461,925) were sourced from the MRC-IEU, while pancreatic cancer (n = 1,896,1,939) was obtained from PanScan1. (Detailed information refer to Supplementary Table 1.)

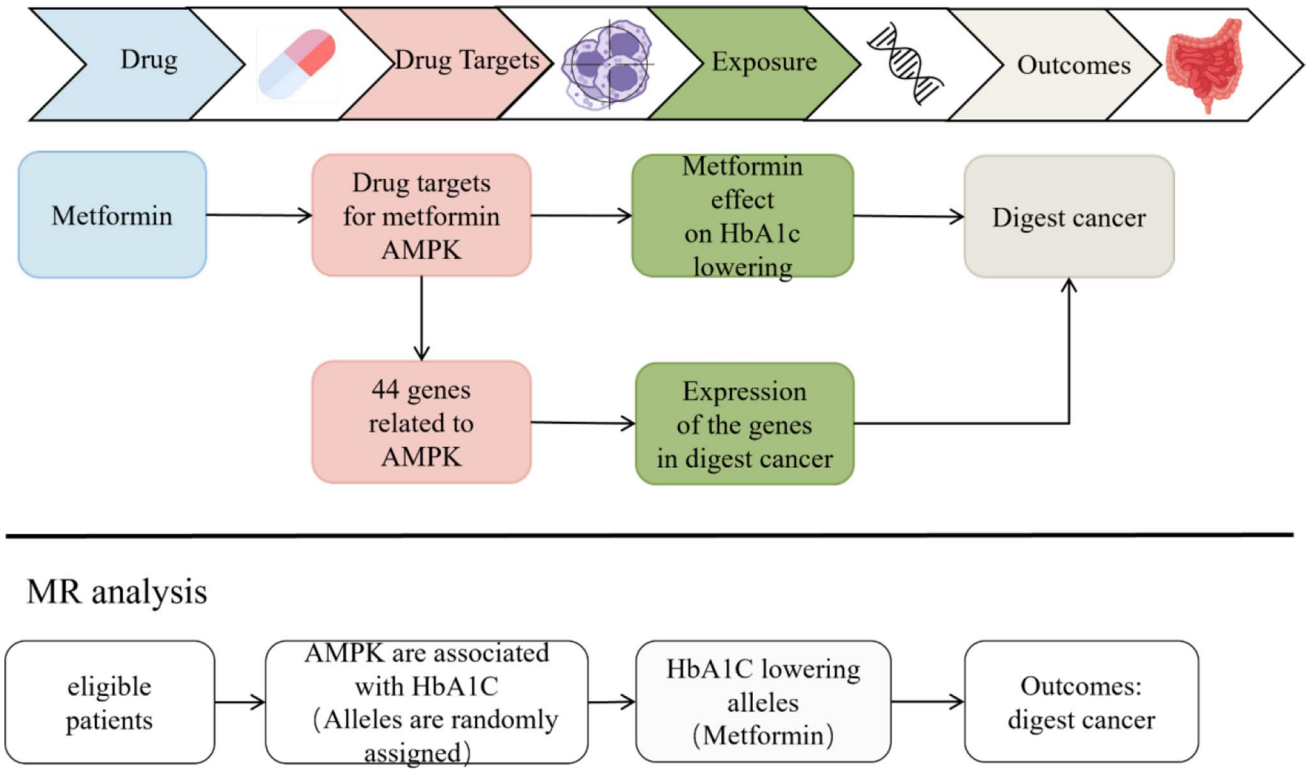

Fig. 1 Study design drawing. This MR Study was designed to determine a causal relationship between metformin (the drug) and metformin-related targets AMPK (the drug target) and digestive tract cancer (the outcome)

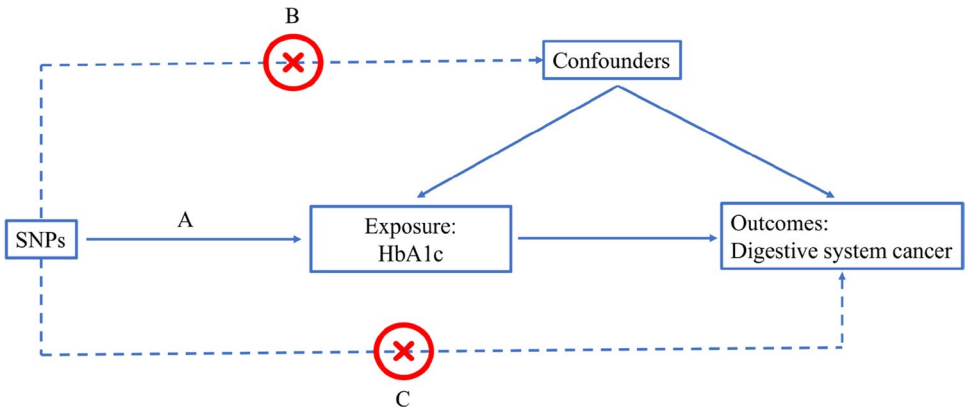

Fig. 2 Diagram for Mendelian randomization (MR). *SNPs* Single nucleotide polymorphisms, *HbA1c* Hemoglobin A1c

2.4 Cell toxicity assay

Huh7 and PLC cell lines were obtained from ATCC and cultured in DMEM supplemented with 10% FBS (Gibco) in a humidified incubator at 37 °C with 5% CO_2. The cells were seeded in 96-well plates at a density of 5000 cells per well. Metformin was added, followed by the CCK-8 solution (C0039, Beyotime Biotechnology), and after 2 h, the absorbance was measured at 450 nm.

2.5 Western blotting

The cells were lysed using RIPA lysis buffer and centrifuged at 12,000 r, 20 min. The supernatant was collected, and total proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 4% skim milk in PBS containing Tween-20 at room temperature for 2 h. Primary antibodies (AMPK 1:5000; 10929-2-AP; Proteintech; p-AMPK 1:2000; 2535 T; Cell Signaling Technology; β-actin 1:1000; GB15001-100; Servicebio) diluted in antibody dilution buffer were incubated overnight at 4 °C. After washing three times with PBST, secondary antibodies (Goat Anti-Rabbit IgG; 1:1000; E-AB-1003; Elabscience; Goat Anti-Mouse IgG; 1:1000; E-AB-1001; Elabscience) were incubated at room temperature for 1 h. Following three additional PBST washes, protein signals were visualized using ECL luminescent solution. Image analysis was performed with ImageJ software (NIH, USA), version 1.53.

2.6 Cell proliferation assay

EdU cell proliferation staining was performed using an EdU kit (BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 488, Beyotime, China). The detailed steps should be carried out in accordance with the manufacturer's protocol.

2.7 Ethics and patient consent

All data were obtained from GWAS studies with ethical review board approval, and all participants provided informed consent.

2.8 Statistical analyses

We employed the Inverse Variance Weighted method (IVW) as the primary approach to obtain MR effect estimates [19]. Weighted Median, MR-Egger, and MR-PRESSO (Pleiotropy RESidual Sum and Outlier) were used as sensitivity analysis methods to assess the robustness of the study results. The Weighted Median method can provide consistent and efficient estimates when over 50% of the information comes from valid IVs [20]. In MR-Egger analysis, the intercept test for the association between exposure and outcome can assess the overall validity of the instrumental variable assumption, where a non-zero intercept suggests potential bias in the IVW estimates [21]. Heterogeneity of the selected instrumental variables was assessed using Cochran's Q test. Additionally, we employed a leave-one-out analysis to determine if the overall estimate was influenced by individual SNPs. Finally, we used the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test to detect horizontal pleiotropy outliers [22]. To rule out false positive results, we calibrated the test level using the bonferroni method. $P < 0.0083$ (0.05/6 cancers) was considered statistically significant. Above statistical analyses were performed using the "TwoSampleMR" packages in R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Student's t-test was used to calculate the differences between two groups, The notation'n = 3'indicates that it represents a minimum of three separate experiments. $P < 0.05$ was considered statistically significant.

3 Result

3.1 SNP selection and validation

We initially selected a total of 44 SNPs. Following MR principles, we subsequently removed two SNPs, namely rs6726126 and rs17834622, as they were associated with potential confounding factors (Colorectal tumors, Neutrophil count, and Lymphocyte count) (detailed information refer to Supplemental Table 2). After performing data harmonization steps (ensuring that the SNP of the exposure and the outcome corresponded to the same allele), the number of retained SNPs for each digestive tract cancer type was as follows: liver and bile duct cancers: 35;

Table 1 Associations between genetically predicted AMPK activation and digestive system cancer by Mendelian randomization

Outcomes	SNPs	IVW		MR-Egger		Weighted median	
		OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P
Liver and bile duct cancer	35	0.994(0.990–0.999)	0.008	0.995(0.986–1.004)	0.249	0.995(0.989–1.001)	0.122
Oesophageal cancer	39	1.003(0.997–1.008)	0.355	1.000(0.988–1.011)	0.966	1.003(0.995–1.012)	0.474
Pancreatic cancer	12	0.206(0.001–39.417)	0.555	83.333(2.000 $\times 10^{-3}$–4.244 $\times 10^{6}$)	0.440	0.756(1.000 $\times 10^{-3}$–1.052 $\times 10^{3}$)	0.939
Colon cancer	13	0.987(0.973–1.001)	0.072	0.999(0.945–1.056)	0.978	0.986(0.962–1.011)	0.247
Malignant neoplasm of rectum	7	1.001(0.985–1.017)	0.859	1.000(0.951–1.053)	0.996	1.001(0.971–1.033)	0.921
Oral cavity cancer	36	1.002(0.998–1.006)	0.272	1.001(0.993–1.010)	0.768	1.000(0.994–1.007)	0.943

AMPK Adenosine 5′-monophosphate (AMP)-activated protein kinase, *SNPs* Single nucleotide polymorphisms, *IVW* Inverse-variance weighted method, *OR* Odds ratio, *CI* Confidence interval

Table 2 Associations between genetically predicted AMPK activation and digestive system cancer in sensitivity analyses

Outcomes	Pleiotropy		Heterogeneity		MR-PRESSO
	Intercept	P	Q	P	P
Liver and bile duct cancer	0.000	0.937	30.043	0.662	0.711
Oesophageal cancer	0.000	0.571	32.334	0.728	0.756
Pancreatic cancer	0.043	0.241	5.015	0.930	0.919
Colon cancer	0.000	0.654	10.113	0.606	0.694
Malignant neoplasm of rectum	0.000	0.960	3.763	0.709	0.807
Oral cavity cancer	0.000	0.806	28.719	0.764	0.770

AMPK, Adenosine 5′-monophosphate (AMP)-activated protein kinase

Esophageal cancer: 39; Pancreatic cancer: 12; Colon cancer: 13; Malignant neoplasm of rectum: 7; Oral cavity cancer: 36; (detailed information refer to Supplementary Table 3).

3.2 AMPK targets on liver and bile duct cancers

IVW analysis indicated a negative association between the genetic prediction of 35 AMPK variants and the incidence of liver and bile duct cancers (OR = 0.994, 95%CI 0.990–0.999; P = 0.008) (Table 1). In the scatter plot of genetic variants'effects on digestive tract cancers, the solid line slope also suggested a negative correlation with liver and bile duct cancers in response to metformin treatment (Supplementary Fig. 1a). Both the Weighted Median and MR-Egger estimated show consistent directional results (Table 1). However, the confidence interval obtained by the MR-Egger method is wider, indicated less precision, which led us to assess horizontal pleiotropy. MR-Egger intercept test and MR-PRESSO test both suggested minimal horizontal pleiotropy ($p < 0.05$), and Cochran's Q test indicated that there is little possibility of heterogeneity bias in our results (Table 2). Sensitivity analysis using the leave-one-out method did not reveal significant outliers in the liver and bile duct cancers analysis, and MR-PRESSO testing also produced similar results, indicating that the overall estimate was not influenced by individual SNPs (Supplementary Fig. 2).

Forest and funnel plots depicting the relationship between metformin treatment target activation and liver and bile duct cancers can be found in Supplementary Figs. 3 and 4, both showing consistent results.

3.3 Effect of metformin on other digest tract cancers

The summary of the MR analysis results can be found in Tables 1, 2, and Fig. 3. IVW analysis indicated that there is insufficient evidence to establish a correlation between genetic predictions related to metformin use and the occurrence of various digestive tract cancers: Esophageal cancer (OR = 1.003, 95% CI 0.997–1.008; P = 0.355), Pancreatic cancer (OR = 0.206, 95%CI 0.001–39.417; P = 0.555), Colorectal cancer (OR = 0.987, 95% CI 0.973–1.001; P = 0.072), Rectal cancer (OR = 1.001, 95% CI 0.985–1.017; P = 0.859), Oral cancer (OR = 1.002, 95% CI 0.998–1.006; P = 0.272)). The MR-PRESSO test suggested a low probability of horizontal pleiotropy ($P < 0.05$), Cochran's Q test indicated that there was unlikely to be

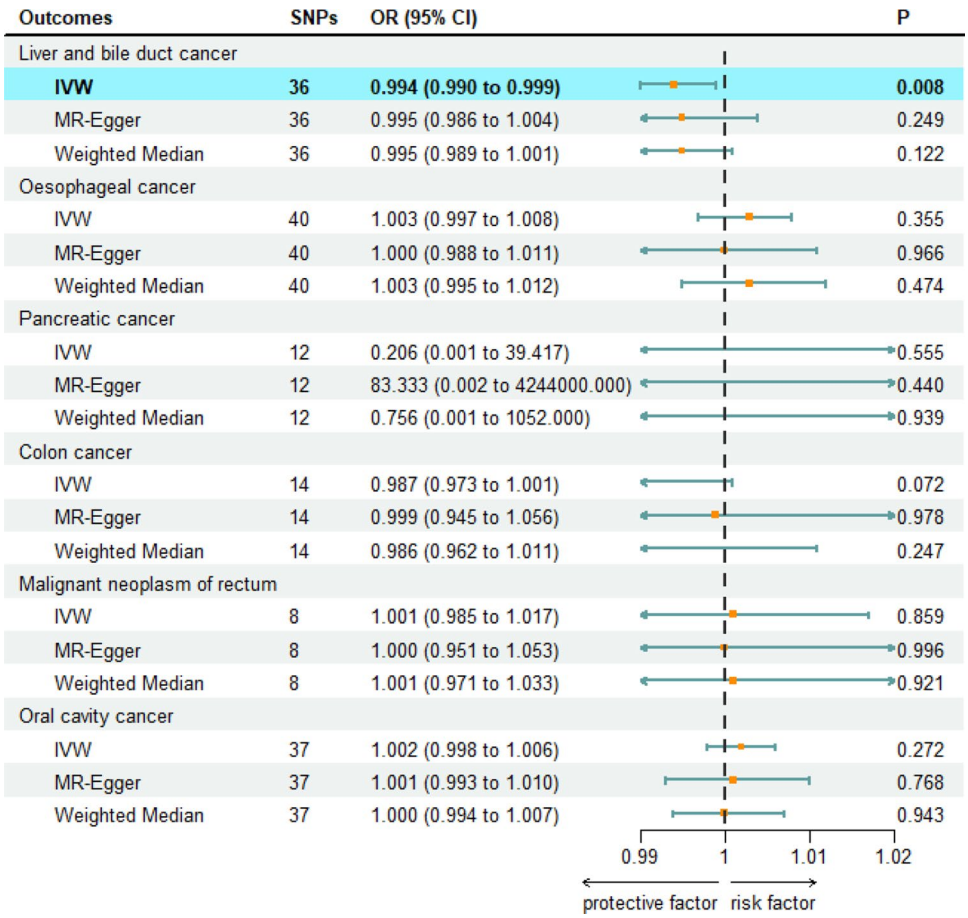

Fig. 3 MR analysis of genetically predicted AMPK activation on digestive system cancers. *MR* Mendelian randomization, *AMPK* Adenosine 5-monophosphate, *AMP* Activated protein kinase

heterogeneity bias in the results, and the leave-one-out sensitivity analysis did not reveal significant outliers (Supplementary Fig. 2).

Scatter plots, forest plots, and funnel plots depicting the relationships between metformin treatment and the effects on other digestive tract cancers can be found in Supplementary-Figs. 1, 3, and 4.

3.4 Metformin inhibits liver cancer cell proliferation via AMPK pathway

To elucidate the critical role of the AMPK pathway in metformin-mediated inhibition of liver cancer progression, we selected two metformin-sensitive liver cancer cell lines, Huh7 and PLC[23], and assessed the effects of AMPK phosphorylation activation on cell toxicity and proliferation (Fig. 4A). In the presence or absence of the AMPK inhibitor dorsomorphin, we treated cells with metformin and found that the activation of the AMPK pathway by metformin was able to rescue cells from dorsomorphin-induced cell death. This further confirms the critical role of AMPK homeostasis in cell survival, and our findings are consistent with previous studies [24] (Fig. 4B, C). Our results demonstrated that metformin suppressed liver cancer cell proliferation and induced cell death via AMPK activation (Fig. 4D, E).

4 Discussion

Our MR study, for the first time, was designed and utilized genetic tools that represent the target of metformin action (AMPK) to analyze the potential causal relationships between metformin and various digestive tract cancers. The study results indicate a significant negative relationship between genetic predictions linked to higher levels of AMPK variation and the occurrence of liver and bile duct cancers. In our study, this conclusion was also confirmed through cell-based experiments. However, there is currently insufficient evidence to suggest relationships between genetic predictions related to AMPK variation and esophageal cancer, pancreatic cancer, colorectal cancer, rectal cancer, and oral cancer.

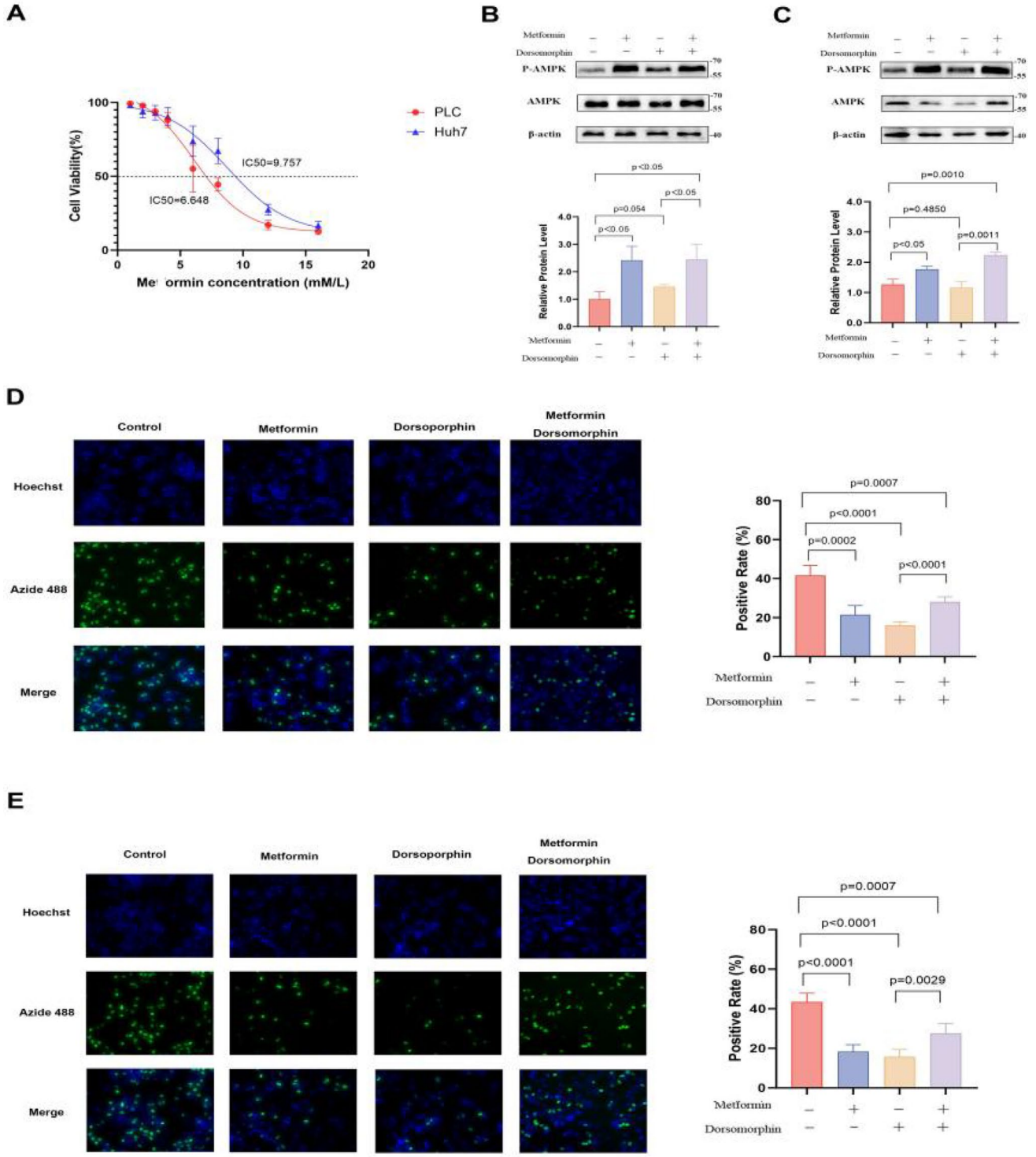

Fig. 4 Metformin inhibits liver cancer cell proliferation via AMPK pathway. **A** IC50 curves for metformin in the Huh7 and PLC cell lines. **B** Western blot analysis of AMPK and p-AMPK expression in Huh 7, along with quantification of their expression levels (n = 3). **C** Western blot analysis of AMPK and p-AMPK expression in PLC, along with quantification of their expression levels (n = 3). The cells were maintained in culture medium containing the corresponding concentration in CCK8 of metformin for 12 h. **D** EdU staining of the Huh 7, with nuclei counter-stained using DAPI (n = 5). **E** EdU staining of the PLC, with nuclei counter-stained using DAPI (n = 5)

In relation to the association between the use of metformin and liver and bile duct cancers, prior case–control studies have yielded similar conclusions. Chaiteerakij, R., in an analysis of 48 patients with intrahepatic cholangiocarcinoma (ICC) who were treated at the Mayo Clinic in Rochester, MN, from January 2000 to May 2010, found that diabetic patients who used metformin had a significantly reduced risk of developing ICC compared to those who did not use metformin. The protective effect of metformin against ICC observed in this study aligns closely with the conclusions drawn in our research [5]. However, the key distinction between our study and the aforementioned observational study lies in our study outcomes avoided confounding bias and reverse causation. MR studies are based on the genetic"randomization"property of one or multiple genetic variants that affect the risk factor, comparing carriers of relevant genetic variations to non-carriers to determine if there is a difference in disease risk. Therefore, the associations we obtained are not influenced by reverse causation and are less likely to be affected by confounding bias, providing a higher degree of confidence in causal relationships [25].

AMPK, through the regulation of cellular signaling and metabolic pathways, coordinates various energy metabolism processes, thereby controlling the growth and progression of cancer cells [26]. Research by Di Matteo, S. and colleagues suggested that metformin can activate the AMPK-FOXO3-related pathway and reverse the mesenchymal and epithelial-mesenchymal transition (EMT) traits in intrahepatic cholangiocarcinoma (iCCA) [27]. In the latest study by Mamedov, M.R., it was found that metformin can identify the abundance of ribonucleoprotein complexes through the AMPK metabolic pathway, enhancing the anticancer activity of cells [28]. All of these findings provide some degree of explanation for our research results.

As mentioned earlier, the primary strength of this study lies in the reduction of confounding factors, and due to the natural"randomness"of allele gene allocation, it can completely avoid time-related biases, such as reverse causation. Additionally, there has been ongoing debate about the relationship between metformin and various digestive tract cancers. We have provided new evidence using genetic and experimental approaches to shed light on this issue.

At the same time, our study has certain limitations and shortcomings, and the interpretation of the conclusions should be approached with caution. We selected AMPK as the target of metformin's action, but existing research has indicated that metformin may impact the occurrence and development of cancers through multiple potential protein targets or pathways, such as Growth Differentiation Factor 15 (GDF-15) [29], mitochondrial complex 1 (MCI) [30], and mitochondrial glycerol 3 (MG3) [31]. We also analyzed these targets, but we were unable to obtain results due to the scarcity of target-related SNPs. Therefore, future analyses focusing on other targets are needed to explore metformin's effects on cancer.

In summary, our study provides a convincing evidence for causal relationships between metformin and digestive tract cancers, particularly in the case of intrahepatic cholangiocarcinoma. However, it is important to note that the conclusion regarding metformin's ability to reduce the risk of intrahepatic cholangiocarcinoma should not be directly interpreted as evidence for its use in the treatment of related cancers. Clinical dosing limitations of metformin must be taken into consideration when translating these findings to potential therapeutic applications.

Acknowledgements We are indebted to all individuals who participated in or helped with this research project.

Author contributions Wang YF participated in the research design, data analysis and writing of the paper, Cao XR, Ren J and Chen R participated in data analysis. Zhang X participated in revising of the paper; Jia YF, Lin T and Liu C provided substantial advice in designing the study and assisting in the division of labor, writing and revising the paper.

Funding Shaanxi Province two-chain integration key special research program (2021LL-JB-06). Shaanxi Province Natural Science Basic Research Program (S2022-JC-QN-1528). Shaanxi Province Natural Science Basic Research Program (2021 JQ-397).

Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Clinical trial number Not applicable.

Competing interests The authors declare no competing interests.

References

1. Chen Y, Ren B, Yang J, Wang H, Yang G, Xu R, You L, Zhao Y. The role of histone methylation in the development of digestive cancers: a potential direction for cancer management. Signal Transduct Target Ther. 2020;5(1):143.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA: A Cancer J Clin. 2023;73(1):17–48.
3. Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. Ann Oncol. 2016;27(12):2184–95.
4. Pernicova I, Korbonits M. Metformin-mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143–56.
5. Chaiteerakij R, Yang JD, Harmsen WS, Slettedahl SW, Mettler TA, Fredericksen ZS, Kim WR, Gores GJ, Roberts RO, Olson JE, et al. Risk factors for intrahepatic cholangiocarcinoma: association between metformin use and reduced cancer risk. Hepatology (Baltimore, MD). 2013;57(2):648–55.
6. Tseng C-H. Metformin and biliary tract cancer in patients with Type 2 diabetes. Front Oncol. 2020;10:587666.
7. Park J-H, Kim Y-H, Park EH, Lee S-J, Kim H, Kim A, Lee SB, Shim S, Jang H, Myung JK, et al. Effects of metformin and phenformin on apoptosis and epithelial-mesenchymal transition in chemoresistant rectal cancer. Cancer Sci. 2019;110(9):2834–45.
8. Wang Q-L, Santoni G, Ness-Jensen E, Lagergren J, Xie S-H. Association between metformin use and risk of esophageal squamous cell carcinoma in a population-based Cohort Study. Am J Gastroenterol. 2020;115(1):73–8.
9. Chen K, Qian W, Jiang Z, Cheng L, Li J, Sun L, Zhou C, Gao L, Lei M, Yan B, et al. Metformin suppresses cancer initiation and progression in genetic mouse models of pancreatic cancer. Mol Cancer. 2017;16(1):131.
10. Wang Q-L, Santoni G, Lagergren J. Diabetes, metformin use, and survival in esophageal cancer: a population-based cohort study. JNCI Cancer Spectrum. 2023;7(4):pkad043.
11. Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH. The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. Int J Cancer. 2012;131(3):752–9.
12. Erkinantti S, Hautakoski A, Sund R, Arffman M, Urpilainen E, Puistola U, Läärä E, Jukkola A, Karihtala P. The association of metformin, other antidiabetic medications and statins on the prognosis of rectal cancer in patients with Type 2 diabetes: a retrospective Cohort Study. Biomolecules. 2022;12(9):1301.
13. Marcano-Bonilla L, Schleck CD, Harmsen WS, Sadr-Azodi O, Borad MJ, Patel T, Petersen GM, Therneau TM, Roberts LR, Brusselaers N. Aspirin, statins, non-aspirin NSAIDs, metformin, and the risk of biliary cancer: a Swedish Population-Based Cohort Study. Cancer Epidemiol Biomark Prevent. 2022;31(4):804–10.
14. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. J Am Soc Nephrol. 2016;27(11):3253–65.
15. Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: a review. Res Synth Methods. 2019;10(4):486–96.
16. Luo S, Schooling CM, Wong ICK, Au Yeung SL. Evaluating the impact of AMPK activation, a target of metformin, on risk of cardiovascular diseases and cancer in the UK Biobank: a Mendelian randomisation study. Diabetologia. 2020;63(11):2349–58.
17. Liang J, Cai Y, Zhang J, Jing Z, Lv L, Zhang G, Zhang R, Liu R, Nan K, Dang X. Metformin treatment reduces the incidence of rheumatoid arthritis: a two-sample Mendelian randomized study. J Clin Med. 2023;12(7):2461.
18. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA. 2017;318(19):1925–6.
19. Lee CH, Cook S, Lee JS, Han B. Comparison of two meta-analysis methods: inverse-variance-weighted average and weighted sum of Z-scores. Genom Inform. 2016;14(4):173–80.
20. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40(4):304–14.
21. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377–89.
22. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(5):693–8.
23. Feng J, Lu H, Ma W, Tian W, Lu Z, Yang H, Cai Y, Cai P, Sun Y, Zhou Z, et al. Genome-wide CRISPR screen identifies synthetic lethality between DOCK1 inhibition and metformin in liver cancer. Protein Cell. 2022;13(11):825–41.
24. Yang J, Zhou Y, Xie S, Wang J, Li Z, Chen L, Mao M, Chen C, Huang A, Chen Y, et al. Metformin induces ferroptosis by inhibiting UFMylation of SLC7A11 in breast cancer. J Exp Clin Cancer Res. 2021;40(1):206.
25. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ (Clin Res Ed). 2018;362:k601.
26. Hsu C-C, Peng D, Cai Z, Lin H-K. AMPK signaling and its targeting in cancer progression and treatment. Semin Cancer Biol. 2022;85:52–68.
27. Di Matteo S, Nevi L, Overi D, Landolina N, Faccioli J, Giulitti F, Napoletano C, Oddi A, Marziani AM, Costantini D, et al. Metformin exerts anti-cancerogenic effects and reverses epithelial-to-mesenchymal transition trait in primary human intrahepatic cholangiocarcinoma cells. Sci Rep. 2021;11(1):2557.

28. Mamedov MR, Vedova S, Freimer JW, Sahu AD, Ramesh A, Arce MM, Meringa AD, Ota M, Chen PA, Hanspers K, et al. CRISPR screens decode cancer cell pathways that trigger γδ T cell detection. Nature. 2023;621(7977):188–95.
29. Gerstein HC, Pare G, Hess S, Ford RJ, Sjaarda J, Raman K, McQueen M, Lee S, Haenel H, Steinberg GR. Growth differentiation factor 15 as a novel biomarker for metformin. Diabetes Care. 2017;40(2):280–3.
30. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577–85.
31. Madiraju AK, Erion DM, Rahimi Y, Zhang X-M, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510(7506):542–6.