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Research progress on neutrophil extracellular traps and hepatitis-to-hepatocellular carcinoma transformation (Review)

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Abstract. Neutrophil extracellular traps (NETs) are web-like structures released by activated neutrophils, composed of DNA, histones and antimicrobial proteins. Although initially discovered for their role in innate immunity, NETs are also closely associated with the pathogenesis of chronic hepatitis and hepatocellular carcinoma (HCC). The present review, which analyzes the latest research from databases such as PubMed and Web of Science, focuses on the formation of NETs, their roles in hepatitis (including hepatitis B, non-alcoholic steatohepatitis and ischemia-reperfusion injury) and their contributions to the initiation, progression and metastasis of HCC. The present review aimed to systematically elucidate the role of NETs in the transition from hepatitis to HCC, with a focus on underlying molecular mechanisms and potential therapeutic implications. The current findings suggest that targeting NET formation or function through inhibition of peptidylarginine deiminase 4, neutrophil elastase, DNase I or related signaling pathways may represent a promising therapeutic strategy to suppress inflammation-driven hepato-carcinogenesis and improve outcomes for patients with HCC. However, further validation is required to translate these findings into clinical applications.

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1. Introduction

Inflammation constitutes a defensive mechanism of the immune system in response to deleterious stimuli, including pathogens, damaged cells or irritant agents (1). Moderate inflammation helps clear infections and repair damage, serving a crucial role in maintaining homeostasis of the body. However, previous studies have indicated that chronic or excessive inflammation may serve a role in oncogenesis (2,3), with >20% of cancers being associated with inflammatory processes (4). Certain cytokines and growth factors involved in the inflammatory response have the potential to enhance cellular proliferation and induce mutations, consequently elevating the risk of cancer development (5).

Globally, liver cancer is the sixth most common cancer and the third leading cause of cancer-associated mortalities. The World Health Organization's International Agency for Research on Cancer reported that in 2022, there were ~870,000 new liver cancer cases and ~760,000 mortalities due to the disease worldwide (6). Hepatocellular carcinoma (HCC) represents 90% of primary liver cell carcinomas and it is the most common type of liver cancer that usually develops in individuals with chronic hepatitis (7).

Neutrophils are increasingly recognized as pivotal contributors to inflammatory infiltration, exerting an inhibitory influence on the cytolytic functions of immune cells. This includes the suppression of natural killer cells, which serve a crucial role in the eradication of tumor cells, as well as lymphocytes and activated T cells (8). Previously, the effect of neutrophils in tumor pathogenesis has garnered notable attention. Emerging research highlights a cytotoxic mechanism

employed by neutrophils operating through the formation of neutrophil extracellular traps (NETs) (9,10). NETs are complex, web-like formations discharged by neutrophils upon exposure to particular stimuli, such as pathogenic infections. These structures consist of chromatin and DNA strands encased in protease granules. Studies indicate that NETs are involved in the initiation and progression of various inflammatory and autoimmune diseases, such as hepatitis B virus (HBV) (11), COVID-19 (12) and rheumatoid arthritis (13). Moreover, NETs contribute to creating a microenvironment that promotes tumor cell adhesion and proliferation, thereby facilitating tumor cell migration and invasion (14). Elevated levels of NETs have been frequently detected in the bloodstream of patients with lung cancer (15), pancreatic cancer (16), bladder cancer (17), HCC (18) and colorectal cancer (19).

Previous research has predominantly concentrated on the roles and effects of NETs in hepatitis (20) and HCC (18). Nevertheless, emerging evidence suggests that NETs are crucial in the progression from chronic inflammation to cancer, especially within the field of hepatology. As a result, the present review aimed to comprehensively investigate the potential mechanisms through which NETs facilitate the transformation from chronic liver inflammation to HCC and to assess their clinical applicability as therapeutic targets for liver cancers. A comprehensive literature review was performed using PubMed and the Web of Science databases, encompassing publications up to October 2025. The search strategy employed key words such as 'neutrophil extracellular traps', 'hepatocellular carcinoma', 'hepatitis', 'liver cancer transformation' and 'inflammation', 'non-alcoholic steatohepatitis', 'non-alcoholic fatty liver disease' and 'hepatic ischemia-reperfusion injury', with an expanded scope through various keyword combinations. The inclusion criteria focused on original research articles and review papers that investigated the role of NETs in the progression from hepatitis to HCC, with a particular emphasis on molecular mechanisms and potential therapeutic strategies. Studies were excluded if they: i) Did not pertain to NETs or the hepatitis-to-HCC transformation; ii) consisted of non-original content such as conference abstracts, reviews or case reports; or iii) lacked complete methodological data or had inaccessible full texts. During the screening process, two reviewers (WYW and CYZ) independently conducted preliminary screening of retrieved literature titles and abstracts based on predefined inclusion/exclusion criteria. Subsequently, full-texts of initially qualified papers were obtained and independently evaluated by the same two reviewers to determine final inclusion. If discrepancies arose between reviewers regarding inclusion at any stage, they would resolve them through mutual consultation. When consensus could not be reached, a third senior researcher (CSG) would arbitrate to ensure objective and fair decision-making. From the ultimately included literature, the two reviewers independently extracted the following information: First author, publication year, study type (*in vivo*/*in vitro*/clinical), research subjects, key findings and NETs-related molecular mechanisms.

2. Formation of NETs

The formation of NETs can be elucidated from three primary perspectives (Fig. 1). The first perspective involves the NADPH-dependent NETosis pathway. In this pathway, the activation of neutrophils by phorbol 12-myristate 13-acetate triggers the activation of NADPH oxidase, leading to the generation of reactive oxygen species (ROS) within the cells (21). ROS are pivotal in the degradation of neutrophil granules and facilitate the release and nuclear translocation of enzymes such as myeloperoxidase (MPO) and neutrophil elastase (NE), which subsequently cleave histones (22). Concurrently, peptidylarginine deiminase 4 (PAD4) is activated, promoting the citrullination of histone 3 (H3). This alteration causes chromatin decondensation and nuclear membrane rupture by decreasing the electrostatic interaction with DNA. The decondensed chromatin translocates to the cytoplasm, where it associates with enzymes such as MPO and NE, and is eventually released extracellularly to form soluble NETs. Throughout this process, neutrophils undergo apoptosis (23).

Conversely, an alternative mechanism of NETosis operates independently of NADPH (24). In this pathway, stimuli such as bacteria or their derivatives, in conjunction with Toll-like receptor (TLR)2, activate neutrophils, subsequently triggering PAD4 activation. This activation results in chromatin decondensation and the translocation of NE to the nucleus. Notably, in this pathway, chromatin, embellished with cytoplasmic and nuclear proteins, is expelled through the formation of nuclear membrane blebs, thereby preserving the nuclear membrane's integrity (25). As a result, neutrophils do not undergo cell death and retain their phagocytic and chemotactic capabilities (26).

In addition, under conditions where mitochondrial oxidative respiration generates notable levels of ROS, neutrophils possess the ability to release NETs that are comprised of mitochondrial DNA rather than nuclear DNA (27). Research using a mouse model of lung metastases from breast cancer has revealed that neutrophils can activate sirtuin 1 through nicotinamide phosphoribosyl transferase secreted by the primary tumor. This activation leads to the opening of the mitochondrial permeability transition pore, thereby facilitating the release of mitochondrial DNA and resulting in the formation of mitochondrial-dependent NETs, which serve a crucial role in this context (28).

3. NETs promote inflammatory-cancer transformation

Previous studies have shown that NETs serve a critical role in the pathogenesis of liver inflammatory diseases and the progression of HCC (11,29-31). The present review also aimed to elucidate the impact of NETs in HBV, non-alcoholic steatohepatitis (NASH), hepatic ischemia-reperfusion injury (HIRI) and HCC (Table I).

NETs promote the development of liver inflammation
HBV. HBV is a critical human pathogen implicated in a range of liver diseases. According to the 'Global Hepatitis Report 2024', ~254 million individuals were infected with HBV and 50 million with hepatitis C worldwide in 2022, with a daily mortality rate of 3,500 attributed to these infections (32). Research performed by Li *et al* (11) demonstrated that in the fulminant viral hepatitis (FVH) mouse model, induced through infection with mouse hepatitis virus strain-3, fibrinogen-like protein 2 (FGL2) interacts with mucolipin-3 to modulate calcium ion influx, thereby triggering autophagy and leading to the formation of NETs.
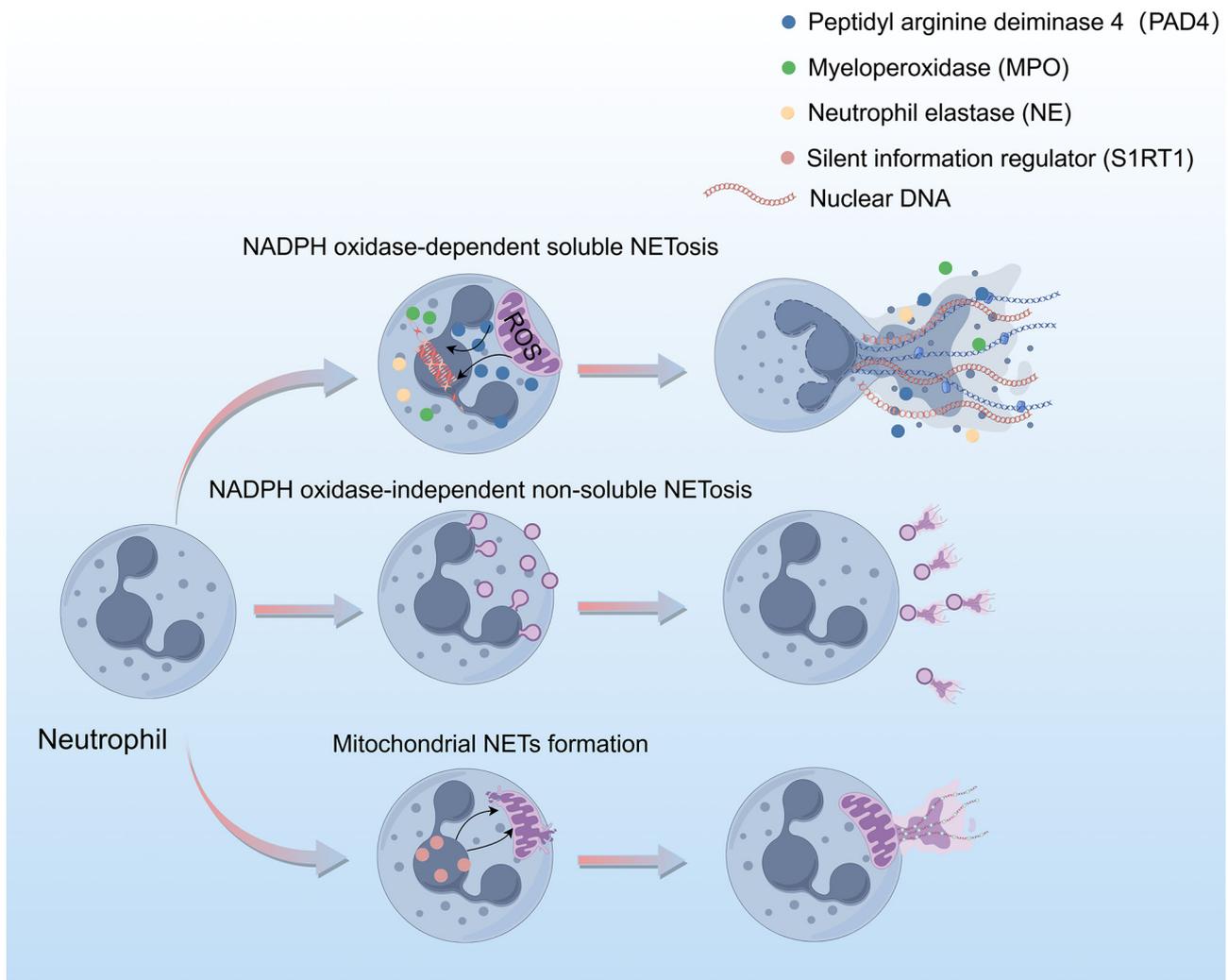
Figure 1. Neutrophils are capable of forming NETs via the NADPH oxidase-dependent NETosis pathway. Furthermore, NETosis can also occur independently of NADPH oxidase activity. Under specific conditions, neutrophils can release NETs composed of mitochondrial DNA, particularly when mitochondrial oxidative respiration produces substantial levels of reactive oxygen species. The figure was drawn using Figdraw (https://www.figdraw.com/static/index. html#/). NETs, neutrophil extracellular traps.

Reduced autophagy was associated with FGL2 deficit and this resulted in a decrease in NET synthesis. Moreover, the depletion of NETs using DNase1 improved liver function and survival rates in FVH mice (Fig. 2). Clinical data further indicate that patients with HBV-induced acute liver injury or acute liver failure exhibit markedly elevated levels of NETs in both plasma and liver tissue compared with patients with chronic HBV and healthy controls. In individuals with chronic HBV infection, there is a reduced release of NETs and an inverse association has been identified between the levels of HBV surface antigen, hepatitis B e antigen (HBeAg), and HBV core antibody and NETs release (33).

Researchers have developed a scoring system based on NETs to predict the 90-day mortality rate in patients with HBV-related acute-on-chronic liver failure, demonstrating superior predictive performance compared with traditional models (34). The research performed by Hu *et al* (35) elucidates that infection with the HBV induces the HBV core and HBeAg proteins to enhance the activity of the mammalian target of rapamycin. This enhancement results in a diminished

autophagic activity in neutrophils and suppresses the activation of ROS-dependent extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathways. Consequently, this suppression inhibits the release of NETs, thereby impairing both innate and adaptive immune responses against HBV and ultimately facilitating the establishment of chronic liver infection (28). Another previous study found that HBV can activate cytotoxic natural killer cells, triggering gasdermin/caspase-8-dependent apoptosis in hepatocytes. Neutrophils selectively accumulate in the apoptotic liver, further inducing NETs, thereby promoting HBV-associated acute-on-chronic liver failure (36).

NASH. NASH represents a more severe form of non-alcoholic fatty liver disease (NAFLD), distinguished by hepatic fat accumulation alongside inflammation and hepatocellular injury (37). Cirrhosis, liver fibrosis and HCC can develop from this illness (38). Neutrophils have been consistently identified within the inflammatory infiltrates associated with NASH (39). In the context of NASH, free fatty acids, such as non-esterified fatty acids, oleic acid and linoleic acid, can

Table I. NETs in HBV, NASH, HIRI and HCC key research summary.

Type of disease	Research models	Key findings	(Refs.)
HBV	i) FVH mouse model (induced by MHV-3); ii) clinical patient samples and hydrodynamic injection model; iii) clinical patient samples, human liver chimeric mouse model of HBV infection, cell co-culture model.	i) FGL2 and MCOLN3 interact to promote autophagy and NETs formation; DNase1 degradation of NETs can improve liver function; ii) HBV core protein and e protein promote chronic infection by inhibiting autophagy and NETs release through mTOR; and iii) HBV activates toxic natural killer cells, causing hepatocytes to die through GSDMD/caspase-8 dependent apoptosis pathway, and neutrophils subsequently form NETs, exacerbating HBV-ACLF.	(11,35,36)
NASH	i) STAM mouse model (STZ + high fat diet at birth); ii) diet-induced LDLR$^{-/-}$ mouse NASH model; vi) NE gene knockout mice and wild-type mice.	i) The formation of NETs is associated with neutrophil accumulation and elevated levels of inflammatory factors, and affects the inflammatory environment of NASH by attracting mononuclear macrophages; ii) the lack of MPO markedly reduced activation of hepatic stellate cells, slowed the development of fibrosis and reduced hepatocyte injury; and iii) the levels of NE were elevated in the livers of wild-type mice with NASH, while the NE-deficient NASH mice showed characteristics such as weight loss, improved blood lipid indexes and reduced inflammatory response.	(29,41,42)
HIRI	i) Mouse liver ischemia-reperfusion model; ii) mouse liver ischemia-reperfusion model and clinical patient samples; iii) machine learning to analyze RNA-sequencing data; and iv) mouse liver ischemia reperfusion model.	i) During hepatic ischemia-reperfusion injury, activated neutrophils release a large amount of ROS and proteases to promote the formation of NETs, thus aggravating liver injury; ii) in the process of liver ischemia and reperfusion, toxic aldehydes, such as acrolein, produced can activate the signaling pathways of NOX2 and p38MAPK in neutrophils to induce the formation of NETs, which further promote liver injury; iii) after HIRI, interleukin-17A promotes neutrophil infiltration and NET formation, which exacerbates liver injury; and iv) HMGB1 induces chromatin decondensation through TLR4 to form NETs and exert inflammatory effects.	(30,46-48)
HCC	i) Clinical patient cohort models; ii) animal models of post-surgical metastasis in simulated liver and PAD4 knockout mice; iii) mouse liver cancer lung metastasis model, mouse *in situ* liver cancer model; iv) *in situ* liver cancer model in mice and clinical patient samples; and v) *in situ* liver cancer model in mice.	i) Preoperative NET levels were associated with patient survival and could serve as a novel biomarker for predicting recurrence-free survival and overall survival in patients with primary liver cancer; ii) liver surgical trauma releases HMGB1, which activates TLR2/4 receptor to recruit neutrophils to form NETs, directly enhancing the ability of tumor cells to migrate, invade and proliferate; iii) NETs release MPO to produce hypochlorous acid, chlorinated layer adhesion protein α5 in the extracellular matrix, activate the integrin β1/FAK signaling pathway, promote the invasion and	(14,56,57, 65,70)

Table I. Continued.

Type of disease	Research models	Key findings	(Refs.)
		metastasis of liver cancer cells and further promote the progression of liver cancer; iv) NETs formed in neutrophils in patients with HCC (particularly metastatic liver cancer) can activate the TLR4/9-COX2 signaling pathway in HCC cells and promote metastasis of liver cancer; and v) intestinal flora imbalance induces intrahepatic metastasis in mouse models by enhancing neutrophil-mediated inflammatory response and inducing excessive production of NETs.	

NETs, neutrophil extracellular traps; HCC, hepatocellular carcinoma; HBV, hepatitis B virus, FVH, fulminant viral hepatitis; MHV-3, mouse hepatitis virus; FGL2, fibrinogen-like protein 2; MCOLN3, mucolipin TRP cation channel 3; GSDMD, gasdermin D; HBV-ACLF, HBV-related acute-on-chronic liver failure; STZ, streptozotocin; LDLR, low-density lipoprotein receptor; NE, neutrophil elastase; NASH, non-alcoholic steatohepatitis; MPO, myeloperoxidase; NOX2, NADPH oxidase 2; HIRI, hepatic ischemia-reperfusion injury; HMGB1, high mobility group box 1; TLR, Toll-like receptor; PAD4, peptidylarginine deiminase 4; FAK, focal adhesion kinase; COX2, cyclooxygenase-2; MAPK, mitogen-activated protein kinase.
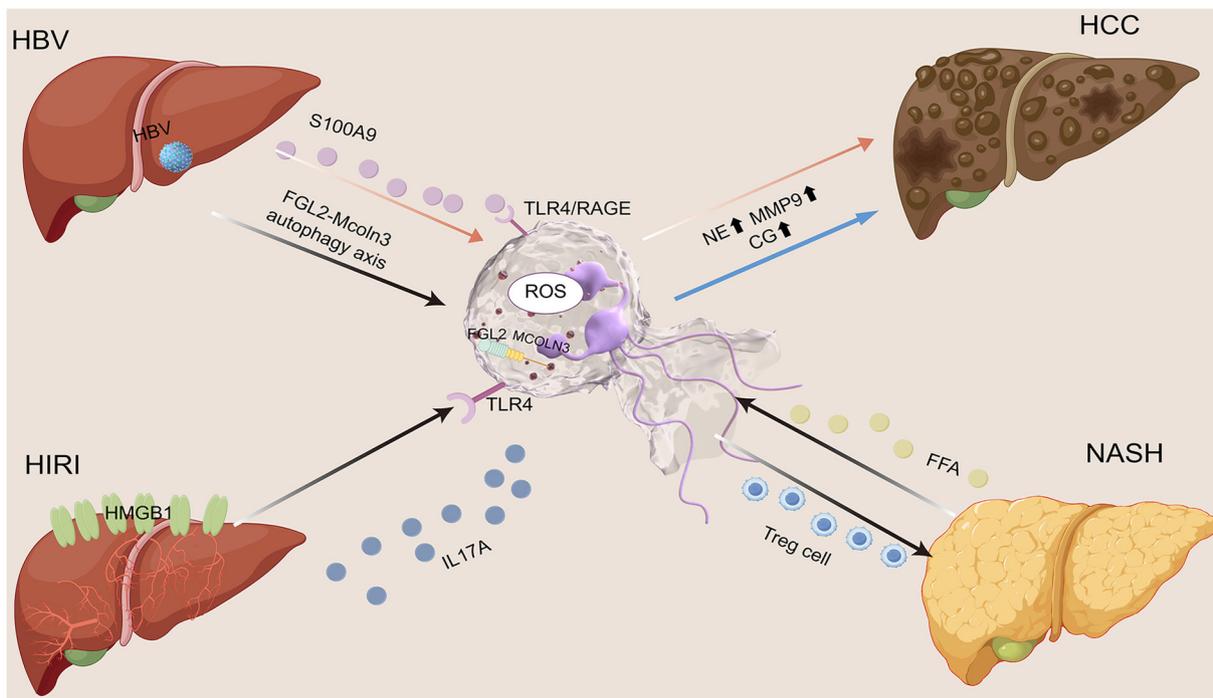

Figure 2. Interplay between NETs and HBV, HIRI, NASH and HCC. Hepatic inflammations such as HBV, HIRI and NASH can promote the formation of NETs by neutrophils; this may therefore promote the development of HCC. The figure was drawn using Figdraw (https://www.figdraw.com/static/index.html#/). NETs, neutrophil extracellular traps; HBV, hepatitis B virus; HIRI, hepatic ischemia-reperfusion injury; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; IL, interleukin; ROS, reactive oxygen species; TLR, Toll-like receptor; MMP9, matrix metallopeptidase 9; NE, neutrophil elastase; Treg, regulatory T cell; FFA, free fatty acids; CG, cholyglycine; MCOLN3, mucolipin TRP cation channel 3; RAGE, receptor for advanced glycation end products; FGL2, fibrinogen-like protein 2; HMGB1, high mobility group box 1.

activate neutrophils to generate NETs, which are prevalent in NASH-affected livers (29) (Fig. 2). Research has indicated that the NETs hallmark proteins MPO and NE are essential for the development of NASH (40). Furthermore, the levels of MPO-DNA in the preoperative serum of patients with NASH are markedly elevated compared with individuals with normal liver function, irrespective of the presence of benign, primary malignant or metastatic hepatic conditions (29) (Fig. 2).

In a STAM (NASH induced by neonatal streptozotocin and high-fat diet) mouse model for NASH, initiated by administering streptozotocin and a high-fat diet to newborn mice, the development of NETs is associated with neutrophil accumulation and increased levels of inflammatory cytokines. This mechanism influences the inflammatory environment of NASH by attracting macrophages derived from monocytes (29). Rensen *et al* (41) showed that lacking MPO markedly reduces the activation of hepatic stellate cells, slows fibrosis development and decreases hepatocyte damage, indicating that MPO is crucial in neuroendocrine tumors and influences the pathogenesis and advancement of NASH. Furthermore, Chen *et al* (42) established NASH models in both NE gene knockout and wild-type mice. The study revealed that NE levels were increased in the liver tissue of wild-type mice with NASH, whereas NE-deficient NASH mice showed weight reduction, improved lipid profiles and decreased inflammatory responses. These findings imply that NE influences ceramide metabolism in the liver, both in living organisms and in laboratory settings, underscoring the crucial involvement of NETs in driving inflammation linked to NASH. According to research, by blocking the development of NETs connected to the production of MPO and citrullinated H3, Tanshinone IIA may reduce inflammation and liver cell death in mice with NASH (43). Some scholars have also found that neutrophil-derived Notch-driven NETs promote the progression of NAFLD to NASH by inducing cellular senescence (44).

HIRI. HIRI is a critical factor that notably impairs postoperative recovery in patients undergoing hepatic surgery. The onset of HIRI rapidly induces an acute inflammatory response, resulting in substantial hepatocellular damage and liver dysfunction, which may progress to multiple organ failure and mortality (45). Research indicates that during HIRI, activated neutrophils release substantial quantities of ROS and proteases, facilitating the formation of NETs and thereby exacerbating hepatic damage (46). At the molecular level, utilizing 101 machine learning combinations to analyze bulk and single-cell RNA sequencing data, researchers identified the involvement of NETs in the pathogenesis of HIRI and early allograft dysfunction. Notably, the administration of C5AR1 antagonists was shown to reduce NETs formation, thereby mitigating hepatic tissue inflammation and enhancing liver function (47).

Furthermore, computational dynamic network analysis has demonstrated that following HIRI, interleukin (IL)-17A facilitates neutrophil infiltration and the formation of NETs, thereby exacerbating liver damage (30) (Fig. 2). In a liver ischemia-reperfusion (I/R) injury model, recombinant high mobility group box 1 protein induced chromatin decondensation via TLR4 to form NETs, which exert pro-inflammatory effects (48). Yazdani *et al* (49) demonstrated that IL-33 released by liver sinusoidal endothelial cells promotes the formation of NETs via the ST2 signaling pathway, thereby exacerbating both sterile inflammation and systemic inflammation within the liver. Additionally, hydroxychloroquine mitigated the formation of NETs by inhibiting TLR9 and suppressing PAD4 expression, thereby alleviating liver I/R injury (46). In the histidine-rich glycoprotein (HRG) mouse model, pretreatment with HRG reduced I/R injury in mice by preventing the production of neutrophils and NETs (50). Additionally, the chemical compounds benzoylphenylurea and Sivelestat sodium were shown to alleviate I/R injury by inhibiting NETs formation (51). Subsequent studies demonstrated that acrolein can trigger neutrophil chemotaxis and stimulate the release of NETs in both *in vitro* and *in vivo* settings (52,53). It was demonstrated that naringin and F-apocynin suppress the production of inflammatory cytokines, activation of the p38MAPK-ERK pathway and apoptotic signaling in rat liver subjected to acrolein exposure and I/R. These compounds effectively suppress acrolein-induced NETs release and mitigate the associated liver I/R injury (54).

NETs promote the development of HCC

NETs are highly expressed in HCC. In HCC, higher NETs formation is associated with raised biomarker levels, such as MPO-DNA in the bloodstream and H3 citrullination in tissue samples. These markers are extensively utilized in clinical trials to identify and quantify NETs formation (55). Kaltenmeier *et al* (56), through a retrospective analysis of serum and tissue samples from patients with liver malignancies, demonstrated that preoperative NETs levels are associated with patient survival and can serve as novel biomarkers for predicting no recurrence and overall survival in individuals with primary carcinoma of the liver. Guan *et al* (57) suggested that IL-8, derived from HCC cells, stimulates the activation of tumor-associated neutrophils to form NETs via the NADPH pathway (Fig. 3). Furthermore, a study assessing the levels of histone DNA, double-stranded DNA and NE in the blood of patients with HCC complicated by portal vein thrombosis (PVT) found that NETs markers were positively associated with the severity of liver disease. Disseminated tumor cells can induce NET formation and expedite the development of PVT (58). Furthermore, empirical evidence suggests that neutrophils producing NETs are predominantly localized near blood vessels within HCC tumor tissues (14). These neutrophils enhance glycolysis by upregulating the pentose phosphate pathway (59). The investigations by Awasthi *et al* (60) demonstrated that glycolysis facilitates the release of NETs via the NADPH oxidase-ROS pathway and also that mitochondrial ROS (mitoROS) are implicated in various aspects of energy metabolism and cellular homeostasis. A study has revealed that neutrophils from patients with HCC exhibit elevated levels of mitoROS and generate NETs enriched with oxidized mitochondrial DNA in a mitoROS-dependent manner (61).

NETs promote the proliferation and growth of HCC. Tumor growth is a multifaceted process influenced by a variety of cellular and humoral environmental factors. Yazdani *et al* (62). found that neutrophil elastase (NE), produced by NETs, activates TLR4 in tumor cells. This activation leads to increased mitochondrial metabolism, which in turn enhances energy production and accelerates tumor cell growth (62) (Fig. 3). Furthermore, by altering the expression of the angiopoietin 2 (Ang-2) gene in endothelial cells, NETs can promote the growth of tumors (63). Ang-2, belonging to the angiopoietin family, is pivotal in altering tumor blood vessels across different pathological states by variably influencing receptor signaling, which in turn enhances tumor progression (64). NETs were found by Tohme *et al* (14) to promote HCC growth and metastasis.
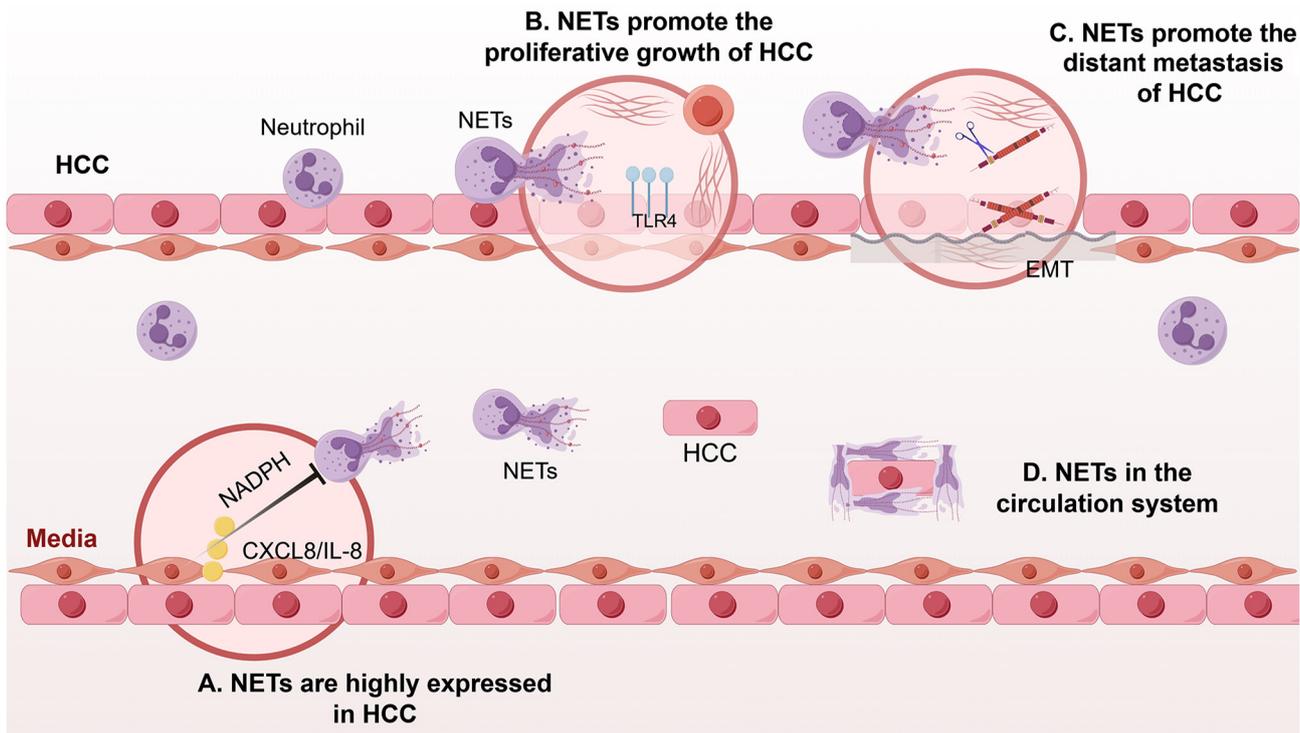
Figure 3. NETs notably contribute to the advancement of liver cancer. NETs are markedly upregulated in hepatic malignancies, where they not only facilitate the establishment of a tumor-supportive microenvironment but also directly enhance the proliferation and metastatic potential of HCC cells. The figure was drawn using Figdraw (https://www.figdraw.com/static/index.html#/). HCC, hepatocellular carcinoma; NETs, neutrophil extracellular traps; EMT, epithelial-mesenchymal transition; TLR, Toll-like receptor; IL, interleukin.

Inhibition of C-X-C motif chemokine ligand 2 can reduce neutrophil recruitment to the tumor site and subsequent NETs formation, thereby impeding the progression of HCC.

NETs promote the metastasis of HCC. Research has demonstrated that NETs facilitate the metastasis of liver cancer. Specifically, NETs contribute to the degradation of the extracellular matrix through surface-bound proteases, including matrix metallopeptidase 9 and NE. This process results in the release of vascular endothelial growth factor, which subsequently enhances tumor invasion and promotes angiogenesis (31). On the other hand, research has indicated that NETs serve a role in promoting the process of epithelial-mesenchymal transition (EMT) within cancer cells. Mechanistic investigations revealed that during NETs formation and release, hypochlorous acid is generated, resulting in the chlorination of tyrosine residues within the LKDYEDLR peptide segment of laminin in the extracellular matrix. This modification of laminin subunit γ-1 by hypochlorous acid can activate the integrin/focal adhesion kinase signaling pathway, subsequently modulating the expression of molecules associated with EMT. This process enhances the migratory and invasive capabilities of tumor cells, thereby advancing the progression of HCC (65) (Fig. 3).

Additionally, NETs can induce the downregulation of VE-cadherin in endothelial cells, compromising the integrity of the tumor vasculature, facilitating tumor infiltration and promoting tumor cell metastasis (66). Within the bloodstream, NETs can encapsulate circulating tumor cells (CTCs) with platelets, creating a robust physical barrier that impedes the interaction between immune cells and CTCs, thereby

facilitating the immune evasion of tumor cells (67) (Fig. 3). Additionally, NETs can augment the adhesive properties of tumors, leading to the capture of CTCs by NETs, which subsequently form stable micro-metastatic foci and progress to larger metastatic sites (68).

In a previous study, it was reported that co-culturing NETs with liver cancer cell lines, specifically Hep3B and CSQT-2, enhances the migratory capacity of these cells. Similarly, the study demonstrated that NETs facilitate inflammatory cell infiltration in the livers of C57BL/6 mice and lead to elevated Ki-67 protein levels in liver tissue following tail vein injection of metastatic tumors (69). This implies that NETs aid in the spread of liver cancer cells throughout the liver (55). Furthermore, research by Guan *et al* (57) revealed a notable increase in NET formation within the neutrophils of patients with HCC, particularly those with metastatic HCC. These NETs possess the ability to ensnare HCC cells, leading to increased resistance to apoptosis and greater invasiveness, thereby boosting their metastatic capabilities. This process is fueled by the TLR4/9-cyclooxygenase-2 (COX2) signaling pathway being activated as a result of HCC cells absorbing NETs. Blocking this pathway can markedly reduce the metastatic potential that NETs confer. Additionally, DNase 1 can directly break down NETs and, when used alongside anti-inflammatory drugs such as aspirin and hydroxychloroquine, it showed notable effectiveness in decreasing HCC metastasis in mouse models (57). A recent study also demonstrated that gut microbiota dysbiosis can promote intrahepatic metastasis in mouse models by enhancing neutrophil-mediated inflammatory responses and leading to the excessive formation of NETs. This implies that

the formation of NETs can be reduced through healthy fecal microbiota transplantation, thereby inhibiting tumor angiogenesis and tissue necrosis, to prevent and treat intrahepatic metastasis of HCC (70).

NETs promote the transformation of hepatitis into HCC. A previous study indicates that >20% of tumors are attributable to chronic inflammation resulting from infections (4). The inflammatory microenvironment can impair the cytotoxic function of human immune cells against tumor cells, enhance extracellular matrix deposition and angiogenesis and ultimately initiate tumorigenesis. Research has demonstrated that *Helicobacter pylori* infection can induce chronic gastritis, with prolonged inflammatory responses potentially facilitating the progression to gastric cancer (71). Similarly, chronic pancreatitis can result in sustained inflammation of pancreatic tissue, thereby elevating the risk of pancreatic cancer (72). Chronic infection with HBV or HCV can lead to persistent hepatitis, with long-term hepatic inflammation potentially progressing to HCC (65). NET development is triggered by inflammatory stimulation, which accelerates the progression of inflammation to malignancy (73). Research has indicated that compared to patients with HCC without HBV infection, the levels of S100A9 protein are markedly elevated in cancer tissues and serum of patients with HBV-associated HCC. Activating neutrophils with S100A9 can induce NETs formation through TLR4/receptor for advanced glycation end products receptors and ROS (65) (Fig. 4).

Animal models have demonstrated that DNase I, ROS scavengers (such as N-acetylcysteine) and S100A9 inhibitors (such as paquinimod) effectively reduce NET formation while notably inhibiting tumor growth and metastasis. This evidence confirms that targeting the 'HBV-S100A9-NETs' axis represents a promising therapeutic strategy (65). Previous investigations uncovered a notable association between serum levels of lipopolysaccharide (LPS) and MPO-DNA in individuals suffering from alcoholic fatty liver disease and alcoholic HCC (74). A previous study performed on mouse models demonstrated that LPS originating from the gut enhances the formation of NETs through the TLR4 signaling pathway, exacerbating liver inflammation and advancing the transition from alcoholic fatty liver disease to HCC (14) (Fig. 4).

In a study performed by van der Windt *et al* (29), NETs generated in NASH mouse models were shown to attract macrophage infiltration, elevate the levels of inflammatory cytokines IL-6 and TNF-α, modify the inflammatory milieu and consequently promote the development of HCC. A study revealed that in NASH-associated liver disease, neutrophils release NETs which, through the TLR4 signaling pathway, alter the metabolic profile of CD4$^+$ T cells. This triggers their differentiation into immunosuppressive regulatory T cells while suppressing the development of cytotoxic effector T cells. The establishment of this immunosuppressive microenvironment ultimately contributes to the progression of NASH-related HCC (75). NETs serve a crucial role in liver I/R injury. Following the initiation of hepatic I/R injury, hepatocytes under stress release damage-associated molecular patterns, which facilitate the infiltration of innate immune cells and subsequently initiate an inflammatory cascade and cytokine storm (76). Upon reperfusion, neutrophils are among the earliest cells to infiltrate the hepatic tissue. Within the liver,

neutrophils may be pivotal in exacerbating tissue damage and promoting tumor progression through the formation of NETs, which contribute to the pro-metastatic cascade (77) (Fig. 4).

Numerous studies have demonstrated that NETs primarily serve a pro-tumor role (78-80). Nonetheless, investigations within alternative cancer models indicate a limited involvement of neutrophils during the initial phases of tumorigenesis. Spiegel *et al* (81) demonstrated that the depletion of neutrophils markedly reduced lung metastasis in models of pancreatic and breast cancer, yet had no discernible effect on the growth of primary tumors. These apparent inconsistencies do not necessarily indicate fundamental contradictions but rather underscore the intricate and dynamic nature of neutrophil biology within the tumor microenvironment. Factors contributing to these variations include distinct triggers of NETosis, such as IL-8 and LPS, heterogeneity in NETs composition and the diverse stages of HCC progression. Despite these complexities, NETs generally exhibit pro-tumorigenic effects during the transformation of HCC. Future research exploring the dual roles of NETs in both promoting and inhibiting tumorigenesis will be essential for the development of targeted therapeutic strategies.

4. Treatment strategies targeting NETs in HCC

HCC, a malignant neoplasm characterized by high global incidence and mortality rates, has attracted considerable attention from the medical community in efforts to advance treatment modalities. NETs are integral to the pathogenesis, progression, invasion, metastasis and thrombus formation associated with tumors, thereby representing a promising target for oncological therapies (82). In light of the relevance of NETs in HCC, researchers have investigated various therapeutic strategies aimed at targeting NETs to enhance treatment efficacy and improve patient quality of life.

The release of NETs can be effectively inhibited through the administration of pharmacological agents such as PAD4 inhibitors (83), NE inhibitors (84) and gasdermin D pore formation inhibitors (85). These agents function by suppressing critical enzymes or molecules integral to NETs formation, thereby diminishing their production.

Enzymatic degradation of the DNA components of NETs is achieved using DNase I, which disrupts the structural integrity of the DNA scaffold. Furthermore, the synergistic application of DNase I with anti-inflammatory agents, including aspirin and hydroxychloroquine, demonstrated efficacy in markedly reducing HCC metastasis in murine models (86). NETs facilitate the capture of HCC cells via the TLR4/9-COX2 signaling pathway, thereby enhancing their invasive and metastatic capabilities. Inhibition of this signaling pathway has the potential to abrogate the metastatic propensity induced by NETs (55).

However, currently, no phase III clinical trials explicitly focus on NETs as a primary target in HCC, and translating NET-targeted therapy from preclinical findings to clinical application faces multiple challenges. Firstly, the etiologies of HCC are diverse and its tumor microenvironment, as well as the specific roles/mechanisms of NETs, exhibit notable heterogeneity. Therefore, it is necessary to identify specific predictive biomarkers for NET-targeted therapy response to help understand treatment outcomes timely and accurately. Secondly, NETs serve dual roles in combating infections and
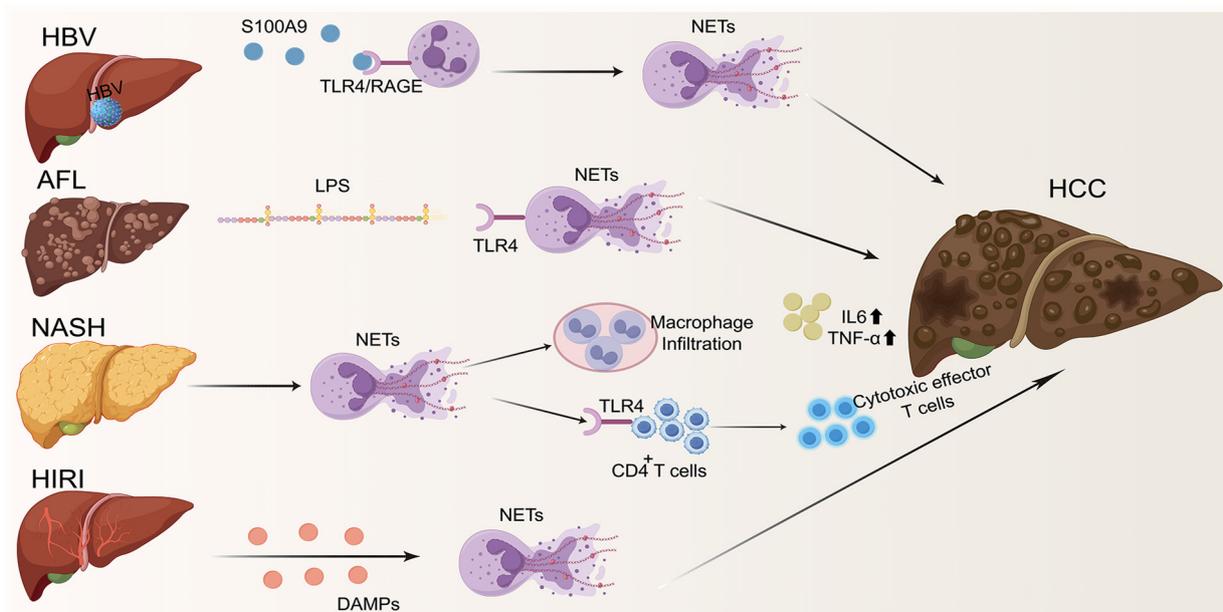
Figure 4. NETs promote the transformation of hepatitis into HCC. NETs can promote the transformation of HBV, AFL, NASH and HIRI to HCC. The figure was drawn using Figdraw (https://www.figdraw.com/static/index.html#/). HCC, hepatocellular carcinoma; NETs, neutrophil extracellular traps; TLR, Toll-like receptor; IL, interleukin; LPS, lipopolysaccharide; HBV, hepatitis B virus; AFL, alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; HIRI, hepatic ischemia-reperfusion injury; DAMPs, damage-associated molecular patterns; RAGE, receptor for advanced glycation end products.

promoting tumor metastasis. Indiscriminate suppression of NETs may increase treatment risks. Determining the therapeutic time window and precisely targeting pathogenic NETs (rather than all NETs) is critical. Additionally, if NET-targeted therapy is applied clinically, urgent issues such as optimizing combination regimens with current treatments, managing overlapping toxicities and screening appropriate indications will need to be addressed.

5. Limitations of current research

Although numerous preclinical findings support targeting NETs for HCC treatment, current research in this field still faces numerous limitations.

Standardization issues in NETs detection and quantification. Current research lacks unified standards for the biomarkers used to detect NETs (such as MPO-DNA complexes and H3 citrullination) and the technical methods employed (such as immunofluorescence, ELISA and flow cytometry) (87). This makes it difficult to directly compare results across different studies, thereby reference intervals, medical decision levels and critical values for NETs levels cannot be established. This hinders the application of NETs as reliable biomarkers in clinical diagnosis and prognostic evaluation.

Translational limitations of animal models. Current research heavily relies on mouse models. However, there are inherent differences between mice and humans in terms of immune system function, liver physiology and the mechanisms of NETs formation (88). For example, mouse neutrophils exhibit different responsiveness to certain stimuli compared with human neutrophils (8). Consequently, the efficacy and safety of various targeted therapeutic strategies that demonstrate

promising results in animal models (such as DNase I and PAD4 inhibitors) still require validation in humans.

Relationship between NETs and HCC treatment resistance. Previous studies have shown that levels of NETs are elevated in patients with breast cancer and animal models with developing drug resistance (89). However, to the best of our knowledge, there are currently no clinical studies on how NETs affect HCC resistance to targeted therapies (such as sorafenib) and immune checkpoint inhibitors. The main reason is that it is currently unclear which specific components within NETs (such as NE, MPO or HMGB1 (High mobility group box-1 protein) act as key effector molecules driving resistance. Additionally, the precise signaling pathways that lead to tumor cell desensitization to sorafenib or immune cell unresponsiveness to programmed cell death protein 1 (PD-1) inhibitors are yet to be elucidated. Due to the majority of current studies being based on preclinical mouse models, it remains unclear whether the levels of tumor-associated or circulating NETs in patients with HCC undergoing treatment with sorafenib or immune checkpoint inhibitors can serve as reliable biomarkers for predicting treatment efficacy and survival outcomes. In conclusion, further investigation is required to understand the mechanisms by which NETs contribute to HCC resistance to targeted therapies, such as sorafenib and immune checkpoint inhibitors, as well as the potential for NET-targeted therapies to overcome such resistance.

6. Summary and outlook

The present study systematically reviewed the critical role of NETs in the inflammatory microenvironment of chronic liver diseases and their transition to HCC. There is growing

evidence that NETs are not only a key component of the liver's inflammatory response but also a critical mediator promoting the 'inflammation-to-cancer' transition. In various liver diseases, including HBV infection, NASH and HIRI, NETs notably drive the initiation and progression of HCC by sustaining chronic inflammation, disrupting immune surveillance and promoting tumor cell proliferation, invasion and metastasis. Targeting NETs formation (such as using PAD4 or NE inhibitors), degrading their structure (such as with DNase I) or blocking related signaling pathways (such as the TLR4/9-COX2 axis) has demonstrated promising anti-HCC effects in preclinical studies, particularly in inhibiting tumor metastasis and improving the tumor immune microenvironment. Additionally, strategies to modulate the gut microbiota to reduce NETs formation offer novel approaches for HCC prevention and treatment.

In summary, the present research underscores the notable role of the neutrophilic microenvironment, specifically NETs, in the progression of HCC. At present, there is no research on the dual application of NETs targeting agents and immunotherapy in the literature. Due to the pivotal functions of NETs in establishing immunosuppressive microenvironments and facilitating therapeutic resistance, the integration of NET inhibitors such as DNase I to degrade NETs, PAD4 inhibitors to prevent their formation or specific antibodies to neutralize their components (90), with current PD-1/programmed death ligand 1 inhibitors is poised to become a novel therapeutic approach in the future. This strategy aims to dismantle the physical and biochemical barriers imposed by NETs, thereby enhancing T-cell infiltration and cytotoxicity within tumors and reversing resistance to immune therapies. As this concept moves toward clinical implementation, ensuring safety remains a critical concern. Future research should prioritize assessing the long-term effects of NET suppression on the body's anti-infective immunity and developing strategies to achieve localized efficacy of NETs within the tumor microenvironment. Identifying specific NET-related biomarkers for patient selection will be essential in advancing this precision combination therapy model.

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Authors' contributions

Conceptualization was performed by CG and HL. YW and CZ wrote the original draft. HW, YZ and YY reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

The patient consents to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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