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Endoplasmic reticulum stress-related regulatory mechanisms in liver disease

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Abstract: The endoplasmic reticulum plays a key role in protein synthesis, peptide chain folding and processing, post-translational modifications, lipid biosynthesis, calcium storage, and detoxification. When endoplasmic reticulum homeostasis is affected by a variety of physiopathological factors, such as nutritional deficiencies, excessive protein synthesis, hypoxia, abnormal endoplasmic reticulum calcium content, lipid overload, oxidative stress, and infections, unfolded or misfolded proteins can accumulate excessively in the endoplasmic reticulum lumen, which can lead to ERS. In recent years, it has been found that the three types of ERS not only are highly correlated with liver diseases but also suppress apoptosis by regulating related pathways to inhibit apoptosis. In addition, the regulation of these pathways and their associated factors is also closely related to immune system diseases and metabolic diseases. In this paper, we review the important roles of endoplasmic reticulum stress-related mechanisms in liver diseases in recent years, which will provide some ideas and insights for future researchers to carry out related studies.

1. Introduction

The endoplasmic reticulum is one of the largest organelles in eukaryotic cells and plays an important role in protein synthesis, processing and transport, in which the process is regulated by a large number of molecular chaperone proteins, glycosylases and oxidoreductases to maintain a certain homeostasis. When endoplasmic reticulum homeostasis is affected by a variety of physio-pathological factors (nutrient deficiencies, over-synthesis of proteins, hypoxia, abnormal calcium content in the endoplasmic reticulum, lipid overload, oxidative stress and infection, etc.), it results in excessive accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum, which in turn triggers ERS[1]. And the cell reduces protein synthesis and decreases the amount of protein entering the endoplasmic reticulum by altering the transcriptional and translational processes; at the same time, it upregulates the expression of molecular chaperones in the endoplasmic reticulum and enhances the protein folding function of the endoplasmic reticulum; and it can also accelerate the degradation process of the unfolded proteins through the upregulation of the expression of genes involved in the endoplasmic reticulum-associated protein degradation pathway. Such adaptive responses generated by the endoplasmic reticulum are collectively known

as the unfolded protein response (UPR)[2]. The UPR needs to be mediated mainly by three transmembrane proteins IRE1a, PERK and ATF6a in the endoplasmic reticulum membrane. Under normal conditions, the molecular chaperone BiP (i.e., GRP78) in the lumen of the endoplasmic reticulum is bound to all three pressure sensing molecules, and when the endoplasmic reticulum is overloaded BiP dissociates from them to activate the three major signalling pathways, IRE1/XBP1, PERK /eIF2a/ ATF4, and ATF6 [3]. The UPR, as a complex signalling network, restores endoplasmic reticulum protein homeostasis and cellular function by increasing protein degradation and decreasing protein synthesis to restore endoplasmic reticulum homeostasis and cellular function. However, chronic ERS induces apoptosis. ERS occurs in the liver when hepatocytes are subjected to lipid metabolism disorders, stress, viral and other damages.

2. Endoplasmic reticulum stress and alcoholic/non-alcoholic liver disease

Non-alcoholic fatty liver disease (NAFLD) is prevalent worldwide and is characterised by an abnormal accumulation of lipids in the liver, often accompanied by insulin resistance, enhanced hepatic inflammation and apoptosis. Recent studies have shown that endoplasmic reticulum stress (ERS) at the subcellular level underlies these characteristic pathologies in the development of NAFLD. Zou Yong found that exercise not only enhances hepatic tolerance to ERS, but also prevents the malignant development of steatosis due to ERS excess and alleviates NAFLD by reducing lipid accumulation, insulin resistance, hepatocyte fat apoptosis, and inflammatory responses[4]. When ERS is activated by PERK-eIF2a-ATF4, ATF4 activates the transcription of the CHOP gene, which contains binding sites associated with unfolded proteins such as (ATF4, ATF6, and XBP1) and can promote apoptosis by regulating the coding of unfolded proteins in the process of apoptosis[5]. Many studies have demonstrated that homocysteine (Hcy) metabolism, oxidative stress, acetaldehyde adducts and other pathways induce elevated Hcy in the blood, a condition known as hyperhomocysteinemia (HHcy). Intragastric alcohol feeding showed a more than fivefold increase in plasma Hcy in mice, and HHcy is a component of several diseases including cardiovascular disease, diabetes mellitus, and alcoholic liver disease[6]. HHcy enhances the production of NF-kB, IL-1b, IL-6 and IL-8, and induces endoplasmic reticulum stress, which could explain many processes of Hcy-promoted cellular injury, such as apoptosis, fat accumulation and inflammation, and the expression of these genes correlates with poor protein folding as well as apoptosis and lipid synthesis, and Hcy activates the GRP78, CHOP, IRE1a, sterol Regulatory Element Binding Protein (SREBP) and JNK pathways[7]. Excessive ethanol intake results in the formation of adducts such as malondialdehyde-acetaldehyde heterodimer and a-hydroxyethyl protein adducts. Studies have shown that malondialdehyde-acetaldehyde adducts increase the induction of IRE1, eIF-2a, GRP78 and CHOP. Furthermore, in a mouse model of acute ethanol intoxication, inhibition of antidiuretic hormone may lead to downregulation of glucose-regulated protein 78 mRNA levels[8]. This suggests a causal relationship between hepatic metabolism of ethanol ERS.

3. Endoplasmic reticulum stress and viral hepatitis

According to the latest Global Burden of Disease report, the morbidity and mortality of chronic hepatitis B have decreased over the past decade, but are still at a high level[9]. HBV infection is a global public health problem, with at least one-third of patients with cirrhosis developing chronic hepatitis B, and a large proportion of patients with chronic hepatitis B eventually progressing to hepatocellular carcinoma. In China, there are about 100 million HBV-infected patients and carriers, and about 500,000 people die prematurely each year from liver failure, cirrhosis, hepatocellular carcinoma and other complications of HBV infection. Although China has made great achievements

in the prevention and control of hepatitis B, the country still has one of the highest numbers of HBsAg-positive people[10]. Currently, the treatment and control of hepatitis B mainly rely on antiviral therapy. In recent years, with the in-depth study of ERS, it has been found that ERS is closely related to viral replication, inflammatory changes and apoptosis in hepatocytes[11]. It is also related to the pathogenesis of viral hepatitis[12-13]. ERS induces a complex homeostatic signalling pathway that maintains physiological homeostasis and protects cells from damage. However, prolonged ERS can lead to apoptosis, which is an important factor in the pathogenesis of many diseases. When viruses infect cells, the endoplasmic reticulum is easily affected by viral replication and synthesis of viral proteins, and a large number of unfolded or misfolded proteins accumulate in the ERS, resulting in ERS. In recent years[14], it has been found that the onset, progression, and regression of a variety of liver diseases are related to ERS, and it has been found that HBV, HCV, and so on, can induce the ERS response in hepatocytes, which is related to viral replication, hepatocyte injury, and the development of hepatocellular carcinoma. HBV and HCV can induce ERS response in hepatocytes and are related to virus replication, hepatocyte damage and liver cancer. In recent years, many scholars have confirmed that HBV itself, HBV-related proteins, as well as inflammatory responses and oxidative stress generated during the disease process can lead to the occurrence of ERS[15-18]. Prolonged or excessive ERS induces apoptosis by inducing a series of biological effect signals from survival to apoptosis, such as CHOP expression, activation of GRP78 and Caspase-12.It has been found[19]. Significant hepatocyte apoptosis and ERS occurred during CCL4-induced hepatic fibrosis (HF), and hepatic GRP78 expression was significantly increased in the HF group of rats. This suggests that the infection process of HBV involves ERS, and ERS induces hepatocyte apoptosis. This in turn indicates that ERS is significantly correlated with the degree of liver inflammation and is involved in the occurrence and development of hepatitis B-related diseases[20].

4. Endoplasmic reticulum stress and liver fibrosis

Liver fibrosis is a reversible process of repair and remodelling after liver injury caused by various factors, and is a common pathological process in various chronic liver diseases[21]. When liver fibrosis occurs, hepatic extracellular matrix (ECM) accumulates excessively in tissues, leading to structural and functional abnormalities in the liver. The endoplasmic reticulum (ER) is an important site for protein synthesis, folding and transport, as well as a key organelle for maintaining calcium homeostasis. Disturbance of the normal function of the endoplasmic reticulum induces endoplasmic reticulum stress (ER stress, ERS), which is a series of pathological processes to restore endoplasmic reticulum homeostasis. PERK is a classical pathway of the UPR, which has multiple roles in liver fibrosis. According to Jo et al[22], ERS increased the expression of dual-specificity phosphatase 5 (DUSP5) in hepatocytes via the PERK/ eIF2a/CHOP pathway, and the induction of DUSP5 was not affected by IRE1 or ATF6 silencing. Overexpression of DUSP5 inhibited extracellular-signal-regulated kinase (ERK) by decreasing its phosphorylation level and increased the level of intracellular activated caspase-3, which ultimately induced hepatocyte death and liver fibrosis. This provides a new molecular basis for ERS-mediated cell death and liver disease. In a study by Lee et al [23], deletion of the transcription factor Krüppel-like factor 10 (KLF10) was shown to be more susceptible to inducing hepatic oxidative stress and ERS in mice fed a high sucrose diet, and the PERK/eIF2a/CHOP signaling pathway was over-activated in hepatocytes of KLF10 knockout mice, resulting in hepatocyte apoptosis, as compared to wild-type mice fed a high sucrose diet. Signalling pathway was over-activated, leading to hepatocyte apoptosis. This suggests a protective role for KLF10 in the progression of hepatic steatosis to fibrosis. He et al[24]showed that the nuclear damage-associated molecular pattern HMGB1 (high-mobility group box 1) could be

released from damaged hepatocytes, activate the TLR4 and RAGE signalling pathways in HSC, and dose-dependently increase the expression of BiP, PERK, and IRE1a to induce HSC activation. In addition, HMGB1 can promote the release of IL-1βand IL-18 inflammatory factors in HSC and induce liver fibrosis.Certain active substances can regulate the PERK pathway and exert antifibrotic activity. gamma-tocotrienol (γT3) was able to reduce the liver function of BiP, CHOP, p-JNK, p-eIF2, p-JNK, and p-eIF2 in mice on high fat and high cholesterol diet along with sucrose drink (HFCS) and methionine and choline deficient diet (MCD), which are the most effective antifibrotic substances.) and methionine and choline deficient diet (MCD) mice, thereby inhibiting ERS and reducing hepatic inflammation and fibrosis by reducing the levels of BiP, CHOP, p-JNK, p-eIF2α and p-p38 proteins in the liver[25]. Liao et al[26]used a small molecule peptide hormone, irisin, to treat CCl4-induced hepatic fibrosis model mice and primary HSC isolated from this model mice, and found that the degree of liver fibrosis and ERS was reduced in the model mice, and the activation of HSC was inhibited in vitro, and found that irisin reduced hepatic nuclear protein through the inhibition of PERK/ eIF2a/CHOP pathway-mediated hepatic nucleoprotein in the HSC HNRNPA1 imbalance to reduce collagen accumulation and alleviate liver fibrosis. These provide new ideas for our treatment of liver fibrosis.

5. Endoplasmic reticulum stress and hepatocellular carcinoma/liver failure

Hepatocellular carcinoma (HCC) is the main form of primary liver cancer and one of the most common fatal malignancies worldwide[27]. The incidence of HCC has gradually increased worldwide in recent years[28], and epidemiological studies have found that the five-year survival rate of HCC is only 18%[29]. Currently, systemic antitumour therapy, especially the combination therapy based on immune checkpoint inhibitors (ICIs), has become the most common and primary treatment for unresectable hepatocellular carcinoma [30-31]. However, hepatocellular carcinoma-related immune-combination therapy is still in its infancy, and research on biomarkers or clinicopathological features predictive of efficacy, as well as related aspects such as the selection of immune-combination regimens and the emergence of clinical resistance, are still being explored. For patients with HCC, the selection of new therapeutic options is necessary. Studies have shown that ERS is closely associated with tumour growth, metastasis and recurrence, and its related genes play a crucial role in tumour progression[32-33]; therefore, researchers generally believe that ERS receptors and their downstream signalling channels play a crucial role in tumour growth, proliferation, and response to chemotherapy, targeted therapies, and immunotherapies[34]. The activation of the UPR by the three downstream general pathways can, on the one hand, reduce ER load and restore ER homeostasis, and, on the other hand, promote malignant cell transformation, such as malignant proliferation, invasion, and metastasis, affecting tumour formation and progression. Numerous tumour-specific genetic, transcriptional and metabolic abnormalities result in an unfavourable local environment, which maintains tumour cells in an ERS state, impairing their function and survival. Despite its deleterious health effects, ER has attracted much attention for its therapeutic applications in cancer treatment, particularly in the induction of apoptosis. ERS-induced UPR has occupied an important place in oncology-related research. In recent years, compounds capable of inducing ERS have attracted attention in HCC research, and the ERS pathway is emerging as a promising target for oncological intervention [35]. An increasing number of scientific studies have highlighted that factors such as adenosine triphosphate (ATP) depletion, abnormal ROS accumulation and radiation exposure exacerbate the ERS response and its induction of apoptosis[36]. In the event that the UPR fails to restore ER homeostasis and alleviate stress, activation of the UPR directs cells towards apoptosis, an apoptotic cascade hypothesised to be associated with the production of Caspase 9 and Caspase-3 in the mitochondrial pathway [37]. For

example, imatinib has been shown to trigger apoptosis in gastric cancer cells via the ERS pathway[38]. It reveals that it exerts its cancer inhibitory effect through the ERS-mediated apoptosis mechanism. It has been shown that ATF6 negatively regulates downstream genes Proteinphosphatase 1H (PPM1H),PPMIF regulates hepatocellular carcinoma cell migration and invasion by dephosphorylating Ribosomal Protein S6 Kinase BL (RPS6KBI), while ATF6 overexpression significantly promoted the proliferation, migration and invasion of hepatocellular carcinoma cells[39]. In order to further confirm that ATF6 is a promoter of HCC, immunohistochemical analysis of ATF6 protein in hepatocellular carcinoma tissues and paracarcinoma microarrays of 124 patients with HCC has demonstrated that ATF6 is closely related to the prognosis of patients with HCC, and that an abnormal increase in the expression of ATF6 is a hallmark of poor prognosis of patients [40].

6. Summary and Prospect

As a big country with hepatitis B, HBV persistent activity in hepatitis B patients can develop into cirrhosis, and patients with hepatitis B cirrhosis who do not receive timely and standardised antiviral treatment will eventually lead to liver failure, portal hypertension and other serious complications, while drug therapy for liver fibrosis caused by hepatitis B mainly focuses on treating the cause of the disease and reducing liver damage by inhibiting viral replication and reducing inflammatory responses. Currently, there is a lack of direct antihepatic fibrosis drugs in the clinic, and more effective treatments are urgently needed to slow down the progression of fibrosis or to promote hepatic fibrosis regression; therefore, the medical demand for the development of more effective and safer antifibrotic drugs remains high. And further research and investigation of how endoplasmic reticulum stress-related mechanisms play a role in liver diseases may provide some new ideas and insights for future researchers when conducting related studies.

References

[1] Zhao S,Liu H M,Tang T S. Endoplasmic reticulum calcium homeostasis and endoplasmic reticulum stress[J]. Chinese Science:Life Sciences,2024,54(08):1318-1326.

[2] Liu B Q,Wang H Q. Research progress of endoplasmic reticulum stress and unfolded protein response [J]. Chinese Journal of Cancer Prevention and Control, 2010, 17(11): 869-872.

[3] Li Z Q, Zhou A R, Tang C S. Progress in the molecular mechanism of endoplasmic reticulum stress response[J]. Chinese Journal of Biochemistry and Molecular Biology,2004,(03):283-288.

[4] Zou, Y., & Qi,Z.(2020). Understanding the Role of Exercise in Nonalcoholic Fatty Liver Disease: ERS-Linked Molecular Pathways.Mediators of inflammation, 2020,6412916.

[5] Thon M, Hosoi T, Ozawa K. Dehydroascorbic acid-induced endoplasmic reticulum stress and leptin resistance in neuronal cells[J]. Biochem Biophys Res Commun, 2016 Sep 16;478(2):716-720.

[6] Ji C, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice[J]. Gastroenterology, 2003 May;124(5):1488-1499.

[7] Esfandiari F, Villanueva J A, Wong D H, et al. Chronic ethanol feeding and folate defificiency activate hepatic endoplasmic reticulum stress pathway in micropigs [J]. American Journal of Physiology—Gastrointestinal and Liver Physiology, 2005 Jul;289(1):G54-63.

[8] Nishitani Y, Matsumoto H. Ethanol rapidly causes activation of JNK associated with ER stress under inhibition of ADH[J]. FEBS Lett. 2006 Jan 9;580(1):9-14.

[9] Collaborators M C O D. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013[J]. Lancet, 2015, 385(9963):117-171.

[10] Shan S, Jia J D. Achievements,opportunities and challenges of hepatitis B prevention and treatment in China[J]. Chinese Journal of Viral Diseases, 2017(1):5-8.

[11] Lee J M. Nuclear Receptors Resolve Endoplasmic Reticulum Stress to Improve Hepatic Insulin Resistance.[J]. Diabetes & Metabolism Journal, 2017, 41(1):10.

[12] Li S, Kong L, Yu X. The expanding roles of endoplasmic reticulum stress in virus replication and pathogenesis.[J]. Critical Reviews in Microbiology, 2015, 41(2):150-164.

[13] Galindo I, Hernáez B, Muñoz-Moreno R, et al. The ATF6 branch of unfolded protein response and apoptosis are

activated to promote African swine fever virus infection[J]. Cell Death & Disease, 2012, 3(7):e341.
[14] Li J, He J, Fu Y, et al. Hepatitis BvirusX protein inhibits apoptosis by modulating endo plasmicreticulumstress response[J].Oncotar get,2017,8(56):96027-96034
[15] Montalbano R, Fazio P D, Quint K, et al. Induction of endoplasmic reticulum-mediated stress pathways in liver cancer cell lines after overexpression of Hepatitis B virus envelope proteins[J]. Zeitschrift F ür - 32 - Gastroenterologie, 2012, 50(1).
[16] Wang H C, Huang W, Lai M D, et al. Aberrant cyclin A expression and centrosome overduplication induced by hepatitis B virus Pre-S2 mutants and its implication in hepatocarcinogenesis[J]. Carcinogenesis, 2012, 33(2):466.
[17] Zhang K. Integration of ER stress, oxidative stress and the inflammatory response in health and disease.[J]. International Journal of Clinical & Experimental Medicine, 2010, 3(1):33-40.
[18] Cho H K, Cheong K J, Kim H Y, et al. Endoplasmic reticulum stress induced by hepatitis B virus X protein enhances cyclo-oxygenase 2 expression via activating transcription factor 4.[J]. Biochemical Journal, 2011, 435(2):431-439.
[19] Wen J J, Xie R J, Yang Q et al. Morphology of endoplasmic reticulum and GRP78 protein expression in hepatocytes of rats with liver fibrosis[J]. Chinese Journal of Pathophysiology. 2011, 27(11):2210-2213.
[20] Liu F. The role of ERS in the pathogenesis and progression of viral hepatitis B [D]. North China University of Science and Technology, 2018.
[21] Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol, 2021 Mar;18(3):151-166.
[22] Jo HJ, Yang JW, Park JH, et al. Endoplasmic reticulum stress increases DUSP5 expression via PERK-CHOP pathway, leading to hepatocyte death. Int J Mol Sci, 2019, 20: 43-69
[23] Lee J, Oh AR, Lee HY, et al. Deletion of KLF10 leads to stress-induced liver fibrosis upon high sucrose feeding. Int J Mol Sci, 2020, 22: 331
[24] He Q, Fu Y, Ding X, et al. High-mobility group box 1 induces endoplasmic reticulum stress and activates hepatic stellate cells. Lab Invest, 2018 Sep;98(9):1200-1210.
[25] Kim Y, Natarajan SK, Chung S. Gamma-tocotrienol attenuates the hepatic inflammation and fibrosis by suppressing endoplasmic reticulum stress in mice. Mol Nutr Food Res, 2018, 62: e1800519.
[26] Liao X, Zhan W, Li R, et al. Irisin ameliorates endoplasmic reticulum stress and liver fibrosis through inhibiting PERK-mediated destabilization of HNRNPA1 in hepatic stellate cells. Biol Chem, 2021 Jan 20;402(6):703-715.
[27] Wen J, Huang Z, Wei Y, et al. Hsa-microRNA-27b-3p inhibits hepatocellular carcinoma progression by inactivating transforming growth factor-activated kinase-binding protein 3/nuclear factor kappa B signalling [J]. Cellular & Molecular Biology Letters, 2022, 27(1): 79.
[28] Deng H F. Efficacy and safety of D-TACE combined with HAIC in the treatment of large or giant hepatocellular carcinoma [D]. Guangzhou: Guangzhou Medical University, 2022.
[29] Wu F, Wei C, Zhang S, et al. The Efficacy of Surgical Resection versus Radiofrequency Ablation for the Treatment of Single Hepatocellular Carcinoma: A SEER-Based Study [J]. Gastroenterology Research and Practice, 2023 Feb 21;2023:1269504.
[30] Ruff S M, Manne A, Cloyd J M, et al. Current landscape of immune checkpoint inhibitor therapy for hepatocellular carcinoma [J]. Current Oncology, 2023, 30(6): 5863-5875.
[31] Jin X, Ma X, Zhao D, et al. Immune microenvironment and therapeutic progress of recurrent hepatocellular carcinoma after liver transplantation [J]. Translational Oncology, 2023, 28101603.
[32] Aghakhani A, Hezave M B, Rasouli A, et al. Endoplasmic Reticulum as a Therapeutic Target in Cancer: Is there a Role for Flavonoids? [J]. Current Molecular Medicine, 2024, 24(3): 298-315.
[33] Zheng S, Wang X, Zhao D, et al. Calcium homeostasis and cancer: insights from endoplasmic reticulum-centered organelle communications [J]. Trends in Cell Biology, 2023, 33(4): 312-323.
[34] Azizi M, Salehi-Mazandarani S, Nikpour P, et al. The role of unfolded protein response-associated miRNAs in immunogenic cell death amplification: A literature review and bioinformatics analysis [J]. Life Sciences, 2023, 31418.
[35] Yuan D. Exploring the role and mechanism of apoptosis induction in hepatocellular carcinoma cells by the traditional Chinese medicine monomer australopithecine based on the regulation of endoplasmic reticulum stress[D]. Changchun University of Traditional Chinese Medicine,2024.DOI:10.26980/d.cnki.gcczc.2024.000790.
[36] Zeeshan H M, Lee G H, Kim H R, et al. Endoplasmic Reticulum Stress and Associated ROS [J]. Int J Mol Sci, 2016, 17(3): 327.
[37] Luna-Marco C, Ubink A, Kopsida M, et al. Endoplasmic Reticulum Stress and Metabolism in Hepatocellular Carcinoma [J]. Am J Pathol, 2022, 193(10): 1377-1388.
[38] Gobin B, Moriceau G, Ory B, et al. Imatinib mesylate exerts anti-proliferative effects on osteosarcoma cells and inhibits the tumour growth in immunocompetent murine models [J]. PLoS One, 2014, 9(3): e90795.
[39] Harrison R E, Bucci C, Vieira O V, et al. Phagosomes fuse with late endosomes and/or lysosomes by extension of membrane protrusions along microtubules: role of Rab7 and RILP [J], Molecular and Cellular Biology, 2003, 23(18): 6494-506.
[40] Zhang J Y. The role of ATF6 downstream gene RAB7A in regulating hepatocellular carcinoma progression and its molecular mechanism [D]. Peking Union Medical College,2024. DOI:10.27648/ d.cnki . gzxhu. 2024. 000391.