Polyploidization is a process by which cells are induced to possess more than two sets of chromosomes. Although polyploidization is not frequent in mammals, it is closely associated with development and differentiation of specific tissues and organs. The liver is one of the mammalian organs that displays ploidy dynamics in physiological homeostasis during its development. The ratio of polyploid hepatocytes increases significantly in response to hepatic injury from aging, viral infection, iron overload, surgical resection, or metabolic overload, such as that from non-alcoholic fatty liver diseases (NAFLDs). One of the unique features of NAFLD is the marked heterogeneity of hepatocyte nuclear size, which is strongly associated with an adverse liver-related outcome, such as hepatocellular carcinoma, liver transplantation, and liver-related death. Thus, hepatic polyploidization has been suggested as a potential driver in the progression of NAFLDs that are involved in the control of the multiple pathogenicity of the diseases. However, the importance of polyploidy in diverse pathophysiological contexts remains elusive. Recently, several studies reported successful improvement of symptoms of NAFLDs by reducing pathological polyploidy or by controlling cell cycle progression in animal models, suggesting that better understanding the mechanisms of pathological hepatic polyploidy may provide insights into the treatment of hepatic disorders.

Key Words: Polyploidization, Hepatocytes, NAFLD, HCC

INTRODUCTION

Polyploidization is a process by which cells are induced to possess more than two complete sets of chromosomes or become polyploid. Whole-genome polyploidy is common among plants, insects, fish, and amphibia; however, in mammals, polyploidy is restricted in certain cell types including hepatocytes and mammary alveolar cells (Wertheim et al., 2013). Polyploidy of these cells results from both nuclear polyploidy, defined as an increase in the amount of DNA per nucleus, and cellular polyploidy, defined as an increase in the number of nuclei per cell (Donne et al., 2020).

Polyploidization was first recognized almost a century ago, and it has been generally considered as a consequence of adaptation during evolution (Comai, 2005). In mammals, the formation of polyploid cells is observed in certain tissues, which are those that undergo development and differentiation, such as heart, placenta, bone marrow, pancreas, and liver (Pandit et al., 2013). Especially, polyploidy in the heart, kidney, and liver was found during regeneration and repair processes (Cao et al., 2017; Lazzeri et al., 2018; Donne et al., 2020). A high frequency of polyploidy has been detected as a consequence of aging in vascular smooth muscle cells, which is accompanied by symptoms of vascular smooth muscle hypertrophy and hypertension (Hixon and Gualberto, 2003). By contrast, unscheduled polyploidization has been observed in pathological conditions. In murine models of non-alcoholic fatty liver disease (NAFLD), the parenchyma of fatty livers displayed alterations of the polyploidization process. Biopsies from patients with non-alcoholic steatohepatitis (NASH) revealed the presence of alterations in hepatocyte ploidy compared with tissue from control individuals (Gentric et al., 2015). Polyploid and aneuploid cells are frequently seen in various solid tumors, probably because proliferating polyploid cells generate aneuploid daughter cells, leading to genomic instability, which is a risk factor for carcinogenesis (Davoli and Lange, 2011).
In this review, we summarize the current understanding of cellular and molecular mechanisms of polyploidization, and the importance of polyploidy in the normal physiology of the liver. In addition, we review the potential roles of polyploidy in pathological conditions of the liver including NAFLDs, and their implications for treatment of these hepatic disorders.

**CELLULAR AND MOLECULAR MECHANISMS OF POLYPLOIDIZATION**

Most polyploidization arises from abnormal cell-cycle processes, except for cell fusion, which is a cell-cycle-independent process. To date, three different mechanisms have been proposed for cell-cycle-mediated polyploidization: endoreplication, mitotic slippage, and cytokinesis failure (Gentric and Desdouets, 2014) (Fig. 1).

First, endoreplication, also referred to as endoreduplication or endocycling, is a cell cycle in which DNA is replicated through alternating gap and S phases, resulting in the formation of a mononucleated polyploid cell (Fox and Duronio, 2013). Trophoblast giant cells are the best-known example of endoreplication in mammals. The cells increase their DNA content to attain a large cell size to form a barrier between the maternal and embryonic tissues (Sher et al., 2013). Accumulation of the cyclin kinase inhibitors such as p57/Kip2 was proposed as a part of the mechanism of endoreplication-mediated cell growth and differentiation of trophoblast stem cells; however, the mechanism has not yet been fully elucidated (Ullah et al., 2008). DNA damage is also responsible for the endoreplication in specific species, cell types, and conditions. For example, UV irradiation causes double-strand breakage-triggered G2/M arrest, which is associated with endoreplication (Radziejwoski et al., 2011). Blockade of mitotic entry after G1/S progression was suggested as an important step leading to endoreplication. Degradation of key regulatory proteins controlling the S phase, such as CDK2, or inhibition of M phase progression factor CDK1, were suggested to cause blockade of mitotic entry (Fox and Duronio, 2013). Second, polyploid cells can be formed after a prolonged arrest in metaphase due to the activation of the spindle assembly checkpoint, which is called mitotic slippage. Subsequently, the perturbed metaphase–anaphase transition causes cell death following mitotic arrest, or mitotic slippage without undergoing anaphase and telophase, which progresses to form mononucleated polyploid cells (Gentric and Desdouets, 2014). Lastly, cytokinesis failure generates binucleated polyploid cells. Cytokinesis is the final step in cell division, leading to the physical separation of a mother cell into two daughter cells. Some cells are programmed to process cytokinesis failure during normal development. For example, hepatocytes exhibit destined cytokinesis failure, which generates multinucleated polyploid cells (Wang et al., 2017).

**POLYPLOIDY ASSOCIATED WITH NORMAL PHYSIOLOGY IN THE LIVER**

The liver is well-known to display ploidy dynamics in physiological homeostasis during its development (Gentric and Desdouets, 2014; Wang et al., 2017). All hepatocytes are diploid at birth, but the cells undergo polyploidization, leading to the gradual heterogeneity of hepatocyte ploidy thereafter. Postnatal binucleation seems to occur during suckling-to-weaning transition (about day 21), which is probably a result of insulin-mediated failure of cytokinesis (Celton-Morizur et
The resulting binucleated hepatocytes successively progress to form mononucleated polyploid hepatocytes (4n) or repeat the process generating multinucleated polyploid cells (2n+2n+2n+2n) to achieve postnatal liver growth. The ploidy level in hepatocytes effectively reaches a plateau at maturity and hepatocytes with octaploidy (8n) appearing in significant numbers of cells during the second and third months after birth (Celton-Morizur et al., 2010). Thereafter, aging-dependent polyploidization occurs during the lifecycle due to cellular senescence (Schwartz-Arad et al., 1989; Celton-Morizur et al., 2010).

The biological importance of polyploidy in hepatocytes has not been understood clearly. The physiological polyploidy of hepatocytes does not show a clear link to functional abnormality of the liver in that prevention of polyploidization by inactivating atypical E2Fs, important regulators of the mitotic and endoreplication cell cycle (vide infra) had no impact on differentiation, zonation, metabolism, or regeneration in the liver (Lammens et al., 2009; Pandit et al., 2012). Meanwhile, some research groups have addressed roles of polyploidy in the liver. Anatskaya and Vinogradov (2007, 2010) showed that gene expression patterns associated with fatty acid metabolism and aerobic respiration were altered in polyploid-associated hepatocytes and suggested a functional role of polyploidy in hepatic adaptation to energy storage, cell survival, and tissue regeneration under stressful conditions. Recently, Richter et al. (2021) analyzed the functional characteristics of 2n and 4n hepatocytes based on a single-nucleus RNA-seq2 method.

Comparing 2n and 4n hepatocytes in the Gene Ontology database and informatics resource showed that 4n hepatocytes were more enriched in pathways involved in lipid, cholesterol, and xenobiotic metabolism, suggesting a potential role of polyploidy in different metabolic potential and position within the hepatic lobule of these cells.

**POLYPLOIDY ASSOCIATED WITH PATHOPHYSIOLOGY IN THE LIVER**

Although hepatocytes in adult liver rarely enter the cell cycle under normal conditions, hepatocytes possess the potential for proliferation in response to hepatic injury from aging, viral infection, iron overload, surgical resection, and metabolic overload such as NAFLDs. Under these circumstances, compensatory proliferation actively takes place, resulting in marked heterogeneity in hepatic ploidy profile. Endoreplication, the specialized cell cycle in which mitosis is skipped, is one of the mechanisms producing nuclear ploidy in injured hepatocytes.
lular turnover and been suggested as an important contributor to liver maintenance during aging (Matsumoto et al., 2021). However, the mechanisms linking polyploidy and aging-induced hepatic dysfunction are not clearly understood and warrant further investigation.

Chronic liver diseases caused by infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) contributes to hepatic adaptation of polyploidy, probably due to cell-cycle defects. HBV X protein (HBx), a crucial viral factor for HBV replication, is assumed to participate in the development of hepatocellular carcinoma (HCC) (Kew, 2011). HBx led to aberrant polyploidi-

zation favoring DNA damage propagation and hepatocyte transformation through DNA-damage-mediated Polo-like ki-

nase 1 (PLK1) activation in HBx transgenic mice (Ahodantin et al., 2019). Furthermore, early transition of G1/S phase and de-

lay of G2/M progression observed in the HBx transgenic hepa-
tocytes eventually led to an increase in the number of mono-
nucleated polyploid cells and decrease in that of binucleated cells, which are representative phenomena of endoreplication (Ahodantin et al., 2019). In the case of HCV, infection with the virus or overexpression of its viral core protein induced defects in the mitotic checkpoint and aberrant chromatin segregation in hepatocytes, which showed frequent hepatic polyploidi-

zation accompanied by reduced Rb transcription and enhanced expression of E2F1 and MAD2 (Machida et al., 2009).

The liver is a major iron storage organ; however, it is also susceptible to injury from iron overload (Bonkovsky, 1991). Especially, iron overload is considered as a potential risk factor for hepatocarcinogenesis (Hino et al., 2021). It has been shown that the process of polyploidi-

zation during carcinogenesis was greatly accelerated in the liver of mice having received iron dextran (Madra et al., 1995). Iron overload in-
duced cyclin D1, a protein involved in the G1 phase of the cell cycle, in a mouse model with carboxyl-iron supplementation or iron-dextran injection, leading to both hepatomegaly and hepatocyte polyploidi-

zation (Troade et al., 2006). Wilson dis-

ease, a genetic liver disease accompanied by hepatic copper overload, has also been associated with increased hepatic polyploidy (Yamada et al., 1998; Muramatsu et al., 2000).

The most widely used model to study liver regeneration and hepatic polyploidy in rodents is partial hepatectomy (PHx) using a surgical resection procedure (Michalopoulos and DeFrances, 1997; Martins et al., 2008). A 70% PHx in-
duced hypertrophy followed by hyperplasia, which caused a decrease in the number of diploid cells and binucleated poly-
ploid cells, but an increase in the number of mononucleated polyploid cells. Mechanisms for pathological and regenerative polyploidi-

zation are quite distinct. According to the amount of resected liver, different patterns of cellular proliferation were observed during recovery of liver mass. Upon 70% PHx, all he-
patocytes entered the cell cycle and rapidly continued through the S phase, but no hepatocytes entered mitosis, resulting in an increase in the number of polyploid hepatocytes. With a resection of >70% of the liver, severe liver injury occurred, and the regenerative capacity of preexisting hepatocytes was significantly impaired (Miyaoka et al., 2012). In this case, liver progenitor cells were activated and progenitor cell-mediated regeneration was the major process in the recovery of liver mass (So et al., 2020). However, after 30% PHx, remaining hepatocytes did not enter into the S phase; instead, hyper-
trophy of hepatocytes contributed to recovery of the original mass (Miyaoka et al., 2012).

HEPATIC POLYPLOIDIZATION IN NAFLDS

NAFLDs refer to a broad pathological spectrum of diseases ranging from simple steatosis to NASH, which can lead to cirr-

rhosis and eventually to HCC (Hardy et al., 2016). The dis-
tinct heterogeneity of hepatocyte nuclear size is one of the unique features of NAFLD (Nakajima et al., 2010; Aravintan et al., 2013). The degree of nuclear enlargement in patients with NAFLD is greater than that in normal individuals, and a larger hepatocyte nuclear area is strongly associated with an adverse liver-related outcome, including HCC, liver transplanta-
tion, and liver-related death (Nakajima et al., 2010; Aravintan et al., 2013). The enlargement of nuclear size in hepa-
tocytes is closely associated with polyploidi-

zation (Watanabe and Tanaka, 1982). In patients with NAFLDs, enrichment of mononucleated polyploid cells was observed throughout the liver parenchyma (Gentric et al., 2015).

In mouse models of NAFLD such as a high-fat diet-induced, methionine–choline-deficient diet-induced, and ob/ob mice, the proportion of mononucleated polyploid hepatocytes was increased, probably due to inefficient cell-cycle progression through the S/G2 phases. Oxidative stress promoted the appearance of highly polyploid cells, and antioxidant-treated NAFLD hepatocytes resumed normal cell division and returned the liver to a physiological state of polyploidy (Gentric et al., 2015). In mice, the liver-specific deletion of SSU72, a protein phospho-
tase involved in sister chromatin separation, led to polyploidi-

zation through endoreplication in chronic liver diseases such as steatohepatitis and fibrosis (Kim et al., 2016). A specific genetic loss of Cdkt1 in the liver yielded increased endoreplication in hepa-
tocytes, which was accompanied by a hepatic phenotype of steatosis and fibrosis in mice (Diril et al., 2012; Dewhurst et al., 2020; Ow et al., 2020). Genetic deletion of E2f7 and/or E2f8 in hepatocytes led to decreased nuclear ploidy and increased G2/M transition, suggesting these genes as central transcription regulators that facilitate DNA endoreplication (Chen et al., 2012). Suppression of E2f8 expression in the liver of zebrafish ameliorated diet-induced obesity and this observation may sup-
port the link between polyploidy and dysregulation of fat me-
tabolism, although its relevance to human NAFLD was not well addressed (Shimada et al., 2015). Recently, a nuclear receptor RORα was demonstrated as a negative regulator of the E2f7 and E2f8 transcription. Deletion of hepatic RORα aggravated symptoms of NAFLD and enhanced hepatic polyploidy (Kim et al., 2022). These molecular factors that regulate hepatic poly-

ploidy in diverse models are summarized in Table 1.

Polyploidi-

zation is closely linked to multiple pathogenic fac-

tors for NAFLDs, such as abnormal lipid metabolism and mito-

chondrial dysfunction. In murine hepatocytes, polyploid nuclei were negatively correlated with the expression of metabolic genes such as ApoA5, Fabp1, Cyp4f15, and Pck1 (Kreutz et al., 2017). A modular biology approach and genome-scale cross-species comparison revealed that gene expression pat-

terns of diploid cells and polyploid cells were different in that genes associated with lipid metabolism were downregulated in polyploid hepatocytes compared with diploid cells (Anatskaya and Vinogradov, 2010). It is noteworthy that expression of genes encoded in the mitochondrial genome has been nega-

tively correlated with nuclear size to a high degree (Miettinen et al., 2014). Hepatocyte senescence resulting from irrevers-

ible cell-cycle arrest correlated closely with fibrosis stage and clinical outcome in patients with NAFLDs. The nuclear area of
hepatocytes increased in patients with progressed fibrosis but was unchanged in patients with improved fibrosis (Aravinthan et al., 2013). Cellular senescence was also reported to act as a driver of age-dependent hepatic steatosis and nuclear enlargement was detected in the hepatocytes in senescence (Ogrodnik et al., 2017). Together, these observations suggest that hepatocellular carcinoma may drive progression of NAFLDs through control of multiple pathogenic factors for the diseases.

### POLYPLOIDY AND HEPATOCELLULAR CARCINOMA

The contribution of polyploidization to development of HCC has been controversial to date. It was proposed that polyploidy provides a genetic buffer from mutation of tumor suppressor genes because polyploid hepatocytes contain extra chromosomes that probably contain an intact allele (Wang et al., 2021). Consistently, it was reported that mouse hepatocytes were more proliferative when in diploidy than in polyploidy (Wilkinson et al., 2019). The tumor-suppressive effect of polyploidy was demonstrated using genetically modified animal models. Diethylnitrosamine treatment of mice with deleted Anih, a gene encoding cytoskeletal scaffolding protein that regulates cytokinesis and might promote tumorigenesis (polyploid model) and E2F7/E2F8 knockout mice (diploid model), showed that the polyploid mouse model was more resistant to the development of HCC after treatment with the hepatocarcinogen (Zhang et al., 2018; Wilkinson et al., 2019). Meanwhile, other investigators have presented evidence for an alternative concept, that is, the tumor-promoting function of polyploidy. Bou-Nader et al. (2020) showed that the percentage of mononucleated polyploid hepatocytes in patients was positively correlated with incidence of HCC and poor prognosis. In a murine model, treatment with diethylnitrosamine increased the generation of polyploid hepatocytes that was accompanied by upregulation of Aurora kinase B (AURKB) (Lin et al., 2021). Further studies are required to address the circumstances and environment that determine the pro- or antitumor effects of polyploidy.

### THERAPEUTIC IMPLICATIONS OF TARGETING PATHOLOGICAL POLYPLOIDY FOR HEPATIC DISORDERS

Due to various infections and metabolic abnormalities, there is a high frequency of developing diseases in the liver. Because of the growing epidemic of risk factors such as overnutrition, the incidence of NAFLD has risen sharply in the past three decades worldwide (Benedict and Zhang, 2017). Recently, the demand for drugs to treat NAFLD and the market size for them have been increasing; however, as yet there is no FDA-approved drug to treat NAFLD. The pathogenesis of NAFLDs is diverse and intertwined with lipotoxicity, inflammatory response, mitochondrial stress, oxidative stress, and fibrogenesis have been developed (Loomba et al., 2021). However, the molecular mechanisms underlying the progression of NAFLDs remain ambiguous and heterogeneous, meaning that the identification of therapeutic targets has been challenging. Recently, many drug candidates targeting pathogenic drivers including lipogenesis, inflammatory response, mitochondrial stress, oxidative stress, and fibrogenesis have been developed (Loomba et al., 2021). Unfortunately, however, they have not achieved their ultimate goals due to insufficient efficacy or unexpected side effects (Neuschwander-Tetri et al., 2015; Ratziu et al., 2016; Mantovani and Dalbeni, 2021). Therefore, identification of new therapeutic targets is warranted to overcome the limits of classical strategies. Although whether polyploidy is a driver of NAFLDs or a consequence of progression of the diseases
is controversial, the mechanism of hepatic polyploidy may pro-
vide new insights into the development of therapeutic strate-
gies for the treatment of the diseases.

Several recent studies in animal models found successful
improvement of symptoms of NAFLDs by reducing pathologi-
cal polyploidy or by controlling cell-cycle progression. Admin-
istration of JC1-40, an RORα activator that induced expression
of E2F7/8, significantly reduced hepatic nuclear polyploidiza-
tion and liver injury in a high-fat diet-induced model of NAFLD
in mice (Kim et al., 2012, 2022). Hepatic steatosis was com-
pletely abrogated in E2F1-deficient mice with diet-induced
NAFLD symptoms (Denechaud et al., 2016). In addition, ge-
netic depletion of gankyrin, an oncogenic regulator of CDK4
and RB, significantly ameliorated liver fibrosis by inhibiting
steatosis and blockade of hepatic proliferation in mice (Daw-
son et al., 2006; Cast et al., 2019). Moreover, administration
of PD-0332991, a small compound that inhibits CDK4, pre-
vented NAFLD development and reversed hepatic steatosis
(Jin et al., 2016). Polyploidy of hepatocytes is associated with
initiation of tumor formation (Lin et al., 2021; Matsumoto et al.,
2021). Pharmacological inhibition of AURKB using AZD1152
reduced nuclear size and tumor foci (Lin et al., 2021). Clearly,
进一步 investigations are necessary to design novel strategies
against pathological hepatic polyploidization to contribute to
the development of therapeutics for diverse hepatic disorders
including NAFLDs.

CONCLUSIONS

Polyploidy is closely related to both physiological and
pathological contexts in the mammalian liver. Recent studies
have now revealed not only the role of hepatic polyploidy in
regeneration and development of the liver, but also its impact
on liver metabolism and cancer. Although there are as yet un-
resolved controversies in the pathological role of polyploidy
during progression of hepatic diseases such as NAFLDs and
HCC, several studies have found beneficial effects of reducing
pathological polyploidy or controlling cell-cycle progression to
improve symptoms of NAFLDs in animal models. Thus, better
understanding the mechanisms of pathological hepatic polyp-
loidization and identification of relevant new targets may provide
insights into the treatment of hepatic disorders.

CONFLICT OF INTEREST

The authors declare no competing interest.

ACKNOWLEDGMENTS

This study was supported by grants from the National
Research Foundation of Korea (2022R1A2C206318 and
2018R1A5A2024425), and Korea Mouse Phenotyping Project
(2014M3A9D5A01073556).

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