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Review Article

Disrupted Regulation of Host Cell Cycle and Its Clinical Significance in Hepatitis C Virus-related Hepatocellular Carcinoma

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Abstract

Growing scientific evidence has suggested the disrupted regulation of host cell cycle proteins in hepatitis C virus (HCV)-associated liver disease. Since the regulation of cell cycle proteins is closely associated with the control of the proliferation and survival of hepatocytes, any alteration in the regulation of these proteins would significantly contribute to the progression of the HCV disease and development of hepatocellular carcinoma (HCC). This mini-review aims to provide an overview of available information on hepatic cell cycle modulations during chronic HCV infection.

Introduction

Even though identified three decades ago, hepatitis C virus (HCV) infection still accounts for substantial cases of liver disease, including cirrhosis (27%) and hepatocellular carcinoma (HCC, 25%) cases, globally.[1,2] The succeeding development and advancement of direct-acting anti-viral agents for treating HCV infection have provided great opportunities to reduce the infection.[3,4] However, early access to diagnosis and treatment remains low. In addition, HCC occurrence and failure of fibrosis regression after sustained viral response are other issues that remain unresolved by direct-acting anti-viral agents.[5,6] Thus, this emphasizes the need to improve the understanding of the molecular mechanisms involved in the pre-cirrhotic stages of HCV-associated diseases, and develop reliable biomarkers that can identify the risk of cirrhosis and HCC development.

The RNA virus of HCV infects and resides in hepatocytes, which are the chief parenchymal liver cells, after evading the host immune response. Furthermore, more than six months of HCV infection can lead to chronic hepatitis C (CHC), which is characterized by an inflamed liver or hepatitis. Even though the inflammatory process is necessary for the removal of virus-infected cells, in CHC, this becomes dysregulated, and the process of fibrosis (the excessive deposition of collagen and extracellular matrix components by stellate cells) is initiated. In addition, the persistent inflammation would also cause hepatocyte damage, which in turn, would induce the proliferation of hepatocytes to regenerate the liver. Thus, in CHC, hepatic cell (hepatocyte) destruction and regeneration, together with inflammation and fibrosis, occurs, leading to the development of cirrhosis and HCC (Fig. 1a).[7,8]

The molecular events in the process of proliferation are closely linked to the regulation of the cell cycle, which is closely correlated to HCC development. A number of literature have revealed that HCV proteins directly modulate cell cycle regulation by targeting tumor suppressors and proto-oncogenes, leading to tumorigenesis.[8,9] The drawback of these studies is that these used transformed cell lines that mostly expressed a single viral protein. Therefore, these cannot represent the actual context of HCV-associated diseases in the liver. Interestingly, studies that involved the immunostaining of cell cycle regulators in archival liver specimens obtained from CHC patients have also described impaired cell cycle progression.[10,11] Even though this data remains scarce, such studies are of great significance, since these represent the disruptions in the pre-cirrhotic stages of CHC inside an HCV-infected liver. The present mini-review briefly describes the cell cycle, and its regulatory checkpoints and disruptions, as reported in CHC patients. In addition, the views of the investigator on how these disruptions contribute to HCV disease progression, and its potential as prognostic and therapeutic markers, were presented.

Cell cycle of hepatocytes and HCV infection

The cell cycle is responsible for imposing tight control on the mo-

Keywords: Hepatitis C virus; Chronic hepatitis C; Cell cycle; Hepatocellular carcinoma.

Abbreviations: CDK, cyclin dependent kinases; CHC, chronic hepatitis C; G1, gap1; G2, gap2; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; M, mitosis phase; MAD2L1, mitotic arrest deficient 2-like protein 1; S, DNA synthesis phase.

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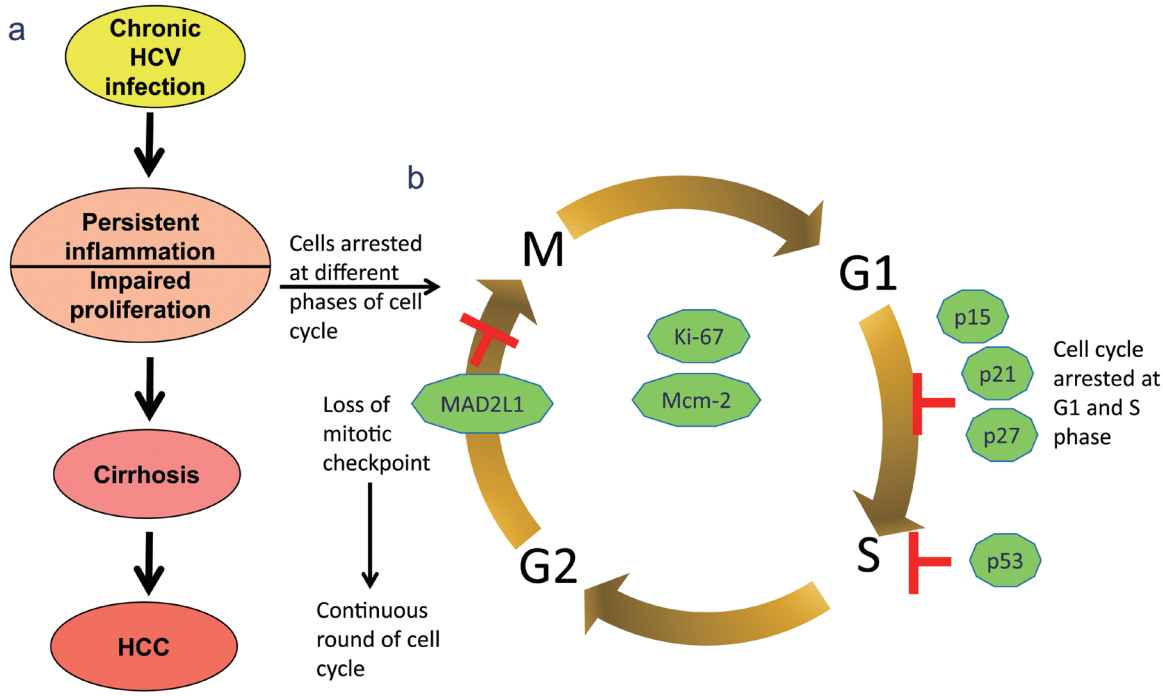

Fig. 1. Involvement of cell cycle disruptions in HCV-associated disease progression. (a) Chronic HCV infection is characterized by the inflammation and impaired proliferation of hepatocytes, which often progresses to cirrhosis and HCC. (b) Impaired proliferation involves the upregulation of cell cycle inhibitors at the G1, S and M phases of the cell cycle, which causes cell cycle arrest. After persistent infection, these disruptions would accumulate, result in the loss of all checkpoints, and lead to continuous rounds of cell cycles. G1, gap1; G2, gap2; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; M, mitosis phase; MAD2L1, mitotic arrest deficient 2-like protein 1; S, DNA synthesis phase.

lecular events of proliferation. The two main phases of the cell cycle are the DNA synthesis phase (S), and chromosomal segregation or mitosis phase (M). These two main phases are separated by interphases or gap phases called, Gap 1 (G1 before the S phase) and Gap2 (G2 before the M phase). Specific proteins called cyclins and cyclin dependent kinases (CDKs) are complexed together during each of these cell cycle phases, making the cell progress to the next phase.[12] In case of any cellular stress or damage, specific inhibitory regulators are activated at the checkpoints between these two phases (*e.g.*, G1/S or G2/M), which stalls the progression to the next phase of the cell cycle.[13] These regulators include tumor suppressor p53, retinoblastoma proteins and CDK inhibitors, such as p15, p16, p21and p27, which inhibit the cell cycle at the G1 and S phases.[14] Mitotic arrest deficient 1 and 2 proteins are localized to unaligned chromosomes and prevent cell division, with a perturbed chromosome assembly at the G2 or M phase.[15] The function of these inhibitory regulators is to provide time for cells to repair the defects. The increase in proliferation of hepatocytes has been suggested for CHC patients, in view of the fact that markers of proliferation, such as Ki67 and minichromosome maintenance protein 2, are elevated in archival liver biopsies of HCV patients.[16,17] However, other investigators have described the disrupted progression of the cell cycle through the G1 and M phases in hepatocytes obtained from CHC patients.[10,11,18,19] On one hand, the disrupted progression of the cell cycle can weaken the regenerative response of the liver to the continuing injury. On the other hand, this can lead to abnormal DNA replication, which increases the risk of transformation and HCC development (Fig. 1b). The following paragraphs discuss the cell cycle disruptions reported in CHC patients in detail.

Regulation of the DNA damage checkpoint (G1 and S phase)

G1/S cell cycle arrest has been reported in hepatocytes in CHC patients due to the increase in expression of CDK inhibitors p15, p21 and p27, as observed by immunohistochemistry (Fig. 1b).[10,18,20] Among these three inhibitors, p21 has a significant correlation with the progression of fibrosis, from stage 0 to stage 4, while the association between p27 and fibrosis was only reported by one study,[18] and this was not confirmed in other studies.[19] The common observation in the above-mentioned studies was the nuclear and cytoplasmic expression of p27. The subcellular localization of these CDK inhibitors plays an important role in their respective functions. For example, nuclear p27 acts as a cell cycle inhibitor and anti-proliferative, while cytoplasmic p27 is a pro-proliferative, and promotes tumor progression.[21] It has been demonstrated that cytoplasmic p27 may act as an oncogenic protein and promote metastasis. In addition, the reduced expression of p27 in HCC has been considered as an independent marker of poor prognosis, which frequently occurs during the advanced stage of HCC.[22] Similarly, the reduced expression of p21 in HCC tissues was observed in the study conducted by Plentz *et al.*[23] The impediment was that these markers have not been extensively and further studied in the pre-cirrhotic stage of the liver disease, hampering the potential to develop as prognostic markers. Another important regulator of the cell cycle at the G1/S phase is p53, which activates in response to any cellular damage, and restricts tumorigenesis by initiating cell cycle arrest and programmed cell death. The immunohistochemical analysis of liver specimens obtained from CHC patients revealed the accumulation of p53 in hepatocytes.[11] Since the expression of p53 is not detectable by immunohistochemistry in normal liver conditions, the investigators suggested that this can either be due to the increase in production of

wild-type p53 or the synthesis of a mutated p53 with greater stability.[11] Indeed, p53 mutations have been reported in HCC patients.[24] It would have been interesting to isolate and sequence the p53 gene from CHC liver biopsies to check for mutations. Overall, these studies suggest the disrupted cell cycle progression in the early stages of the HCV disease, which might be a consequence of cellular stress, or a viral strategy employed to generate a favorable environment, and use the cell machinery for its own replication.[25] In addition, an arrested cell cycle would impair the regenerative response of the liver to the ongoing injury during HCV infection.

Regulation of the mitotic checkpoint

Flaws in chromosomal segregation are a common characteristic of liver tumor cells, indicating the possible role of M phase deregulation in the pathogenesis of HCC.[26] The recent study conducted by the investigator revealed that the immunopositivity of MAD2-like 1 (MAD2L1) protein was identified in nearly 80% of CHC patients. In addition, it was observed that the MAD2L1 expression was significantly associated with the stages of fibrosis (1–4).[19] The MAD2 family of proteins plays a significant role in the mitosis step of cell division and subsequent chromosomal segregation.[27] Any dysregulation in its expression level would increase the likelihood of mitotic errors and aneuploidy, which often leads to cancer.[28,29] The further support to this hypothesis came from an *in vitro* study that demonstrated the increase in MAD2 protein expression in HCV expressing hepatic cell lines, which led to mitotic arrest and the development of chromosomal polyploidy.[30] Interestingly, the MAD2L1 expression has already been reported in HCC tissues through western blot, and this has been shown to have a positive correlation with the size and invasion of tumors.[31] Thus, the presence of MAD2L1 in the pre-cirrhotic stages of HCV infection reflects some of the earlier defects in the mitotic checkpoint, which might later lead to mutations and the subsequent development of HCC. Further studies should be conducted to determine the prognostic potential of MAD2L1 immunostaining in archival samples, which can be used as a tool to identify CHC patients with high risk of developing HCC.

The main hindrance to continuing such studies was the decline in liver biopsy procedures during the last decade due to the availability of alternate technologies for evaluating liver histology.[32]

Conclusion

The cell cycle disruption in different stages of CHC might be a consequence of cellular stress, or a viral strategy to keep the cells alive and use their machinery. Understanding these disruptions in the early stages of CHC using archived liver biopsy specimens can help in the prognosis and improvement of therapeutic treatments for CHC and its sequel.

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Conflict of interest

The author declares no conflicts of interest.

Author contributions

Saira Khalid is the sole author of this manuscript.

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