Cancer drugs for hepatitis B treatment: what do we know?

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HBV infection & its consequences

Currently, persistent chronic HBV infection is an incurable disease. The infection specifically targets the liver and it has affected an estimated 2 billion people worldwide. HBV infection is associated with an increased risk for patients of developing liver cirrhosis and hepatocellular carcinoma (HCC) [1]. The majority of people infected with HBV are able to control and suppress viral replication, but nonetheless they are still at higher risk of developing HCC compared with the general population. A proportion of patients develop a persistent active infection and associated hepatitis that can progress, if left untreated, to cirrhosis and HCC. HBV infection is responsible for 50 and 33% of deaths related to liver cancer and cirrhosis, respectively [2]. As a result, HCC is now one of the leading causes of cancer-related deaths worldwide.

Current antiviral therapies for chronic HBV infection

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carriers of the virus [1]. The vaccine has no efficacy in treating people that have already acquired infection. Approved therapies for chronic HBV infection include pegylated IFN- α , which is an immunomodulatory drug, as well as nucleotide or nucleoside analogs (NUCs) that act as reverse transcriptase inhibitors blocking viral replication. IFN- α therapy is associated with low response rates and its use is associated with several side effects. Although NUCs are potent drugs that are well tolerated and show excellent efficacy in suppressing viremia, they are not capable of inhibiting hepatitis B s-antigen or e-antigen (HBsAg, HBeAg) production. Treatment with some NUCs, such as lamivudine, is often associated with the development of drug-resistant HBV mutants. Fortunately, resistance has not yet developed to the latest generation of NUCs.

Nevertheless, none of the present therapies are capable of eliminating all HBV genome from the liver and consequently viral rebound can occur following cessation of treatment. Even in people that suppress viremia, spontaneously or following drug treatment, viral rebound can occur if patients become immunosuppressed.

KEYWORDS

- apoptosis • cancer drugs • HBV
- immune modulation

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Covalently cccDNA, an episomal replicative form of HBV-DNA, persists in the nucleus of infected hepatocytes, triggering relapses in the aforementioned situations. Therefore, life-long daily treatment with NUCs is the current recommendation [3].

The ultimate goal of HBV treatment is the elimination of cccDNA, the replicative template and main driver of persistent active infection. There is an urgent and unmet need for new therapies that eliminate all persistent HBV genome capable of reactivating viremia. Such treatments would mitigate the morbidity and mortality associated with chronic HBV infection.

Progress in defining novel therapeutic for chronic HBV infection

Novel antiviral drug approaches include the attempt to inhibit HBV entry into hepatocytes and hence abrogate the spread of HBV throughout the liver. A very promising study is a Phase IIa clinical trial using a recombinant myristoylated form of the HBV pre-S peptide (Myrcludex B). In preclinical models this therapeutic was efficacious in limiting infection [4]. The peptide competitively blocks binding of HBV to the hepatocyte sodium taurocholate cotransporting polypeptide (NTCP), which is the receptor for HBV entry [5].

Another research focus is the development of anti-HBV drugs that target different points in the HBV life cycle. Current intriguing approaches include targeting HBV capsid protein assembly [6]. The most promising candidate is ‘NVR 3-778’ developed by Novira Therapeutics [7]. This compound inhibits HBV replication by inducing the assembly of defective viral capsids and is currently in Phase I clinical trials. A novel RNAi approach, also in Phase I clinical trials, was developed by Arrowhead Research Corporation. Cholesterol-conjugated siRNAs (ARC-520) are used to target multiple HBV mRNAs. This potential therapeutic directly targets the HBV lifecycle and may indirectly promote an adaptive immune response against HBV.

An alternative promising approach is to promote or target host cell immune modulators that can aid in the specific degradation of HBV cccDNA. *In vitro* studies have examined the combination of IFN- α treatment with lymphotoxin- β receptor agonists in an effort to promote cytidine de-amination and APOBEC3A/APOBEC3B activity [8]. Further preclinical studies are required to validate this therapeutic option.

Anticancer drugs as anti-HBV drugs

A novel strategy to eradicate HBV-infected cells, and the focus of this article, is the concept of killing HBV infected cells, thus destroying the reservoir of cccDNA. At our disposal now, is a huge array of anticancer drugs that target specific cellular pathways to promote the death of aberrant or abnormal cells. A class of immune modulatory compounds currently tested in cancer immunotherapy is PD-1 inhibitors [9]. PD-1 is a cell surface receptor of the immunoglobulin superfamily expressed on T cells and B cells. Stimulation of PD-1 by its cognate ligand called PD-L1, present on the surface of many cell types, prevents the activation of the immune system by inhibition, or the induction of apoptosis, of activated T cells. PD-1 antagonistic antibodies are used to augment tumor-specific T-cell responses by preventing PD-1 and PD-L1 interactions between immune and tumor cells.

Several studies have tried to translate this discovery to HBV infection [10,11]. PD-1 antagonist antibodies may potentially promote the activity of virus-specific T cells and assist in the immune-mediated elimination of HBV infected cells. Promising preclinical data have been obtained in mouse and woodchuck models of HBV infection.

HBV-infected hepatocytes share several features with cancer cells (including HCC), in particular the activation of many cellular stress responses. Cells under stress normally undergo apoptosis, but many abnormal cells appear to abrogate the activation of this cell death program. A number of viruses have developed different strategies to manipulate apoptotic signaling. Some viruses promote apoptosis of host cells in order to facilitate pathogenesis. For example, the HIV-1 causes the death of CD4⁺ T cells through various mechanisms to escape adaptive immune responses [12]. Other viruses successfully circumvent induction of apoptosis and promote survival of infected cells in order to allow viral replication and subsequent dissemination. For instance, Epstein–Barr virus (EBV) and some human herpes virus code for functional homologs of host Bcl-2 proteins that normally function to inhibit the activation of the apoptotic program. Other viruses, such as cytomegalovirus (CMV) express active caspase inhibitors to prevent apoptosis [13].

Several studies have examined the role of host apoptotic regulatory proteins during HBV infection. It was demonstrated that HBV replication does not seem to sensitize infected cells to apoptosis [14]. Moreover, HBV core protein inhibits

apoptosis mediated by Fas, a TNF death receptor family member [15], as well as TNF-related apoptosis-inducing ligand, TRAIL [16]. Both of these death ligands are involved in the regulation of the immune system and killing of abnormal cells.

An additional extrinsic death-signaling pathway that HBV has adapted to is TNF signaling. Under steady-state TNF signaling drives NF- κ B activation, which promotes cellular activation and survival and is advantageous for viral replication [17]. Cellular inhibitor of apoptosis proteins (cIAPs) are a family of intrinsic adaptor molecules involved in regulating and promoting TNF-mediated cell survival signaling. It was shown that HBV-infected cells express high levels of cIAPs, thus promoting a beneficial environment for replication [18]. Additionally, it was recently demonstrated that cIAPs, predominantly cIAP1/2, inhibit the clearance of HBV [19]. Therefore these cIAP molecules may represent important candidates that can be targeted with small compound drugs.

IAPs themselves are regulated by Smac/Diablo, an endogenous protein activated through cytochrome-c release from the mitochondria, once apoptosis is initiated in a cell. Drug inhibitors of cIAPs are a group of small molecule compounds called Smac mimetics, since they mimic the ability of Smac to antagonize the function of IAPs. The therapeutic potential of inducing TNF-mediated apoptosis has been studied in clinical cancer trials [20].

It was recently demonstrated that this approach, of promoting TNF-mediated induction of apoptosis, can be repurposed to induce killing of HBV-infected cells [21]. Using immunocompetent mouse models of chronic HBV infection, the clinical-stage Smac mimetic 'birinapant' by TetraLogic Pharmaceuticals was used to antagonize cIAPs and promote TNF-mediated eradication of HBV-infected cells. The drug preferentially kills infected cells and leaves non-infected cells unharmed. This is probably because TNF is primarily released by immune cells, which preferentially aggregate around areas of infection, and because HBV infected hepatocytes express high levels of the TNF receptor TNFR1. This study

revealed for the first time that the application of Smac mimetic molecules is a promising new strategy to clear HBV from the host.

Conclusion

Having an array of potent novel anticancer therapies at hand provides us with a great opportunity to directly test their therapeutic potential to treat chronic HBV infection. The repurposing of anticancer compounds to selectively kill infected cells is a novel treatment strategy to tackle the health burden caused by HBV. Using this knowledge and translating these drugs to treatments for chronic HBV infection represents a challenging assignment for the future.

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