Antiviral Chemistry and Chemotherapy
2017, Vol. 25(2) 53–57

DOI: 10.1177/2040206617701372
journals.sagepub.com/home/avc
SAGE

Metabolic reprogramming during hepatitis B disease progression offers novel diagnostic and therapeutic opportunities

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Abstract

Metabolic remodeling occurs in immune cells during an infection. Host cells must upregulate energy production for growth, proliferation, and effector functions to limit the damage imposed by pathogens. One example, the hepatitis B virus, induces hepatic injury in human hepatocytes through dysregulation of aerobic glycolysis and lipid metabolism. Increased glycolytic metabolism mediated by elevated expression of Glut1, glucose influx, and lactate secretion is associated with this Warburg phenotype, a classic metabolic signature also observed in cancer cells. This article brings into focus the tight interaction between HBV infection and metabolic dysfunction and how these processes facilitate the progression of end-stage liver diseases, such as hepatocellular carcinoma. We also provide evidence and models by which other viruses such as HIV and Zika disrupt their host metabolic machinery. The emergence of the immunometabolism field provides novel opportunities to take advantage of intermediary metabolites and key metabolic pathways for diagnostic and therapeutic purposes.

Keywords

Hepatitis B virus, hepatitis C virus, HIV, immunotherapy

Introduction

Chronic hepatitis B infection affects more than 300 million people worldwide and is a leading cause of liver failure and cancer. Although current treatments for chronic HBV suppress viral replication and reduce the risk of liver cancer and end-stage liver disease, it does not constitute a ''sterilizing cure.'' Thus, treatment interruption may result in resurgence of viral replication and flare of hepatic diseases.

The emergence of the immunometabolism field has reinvigorated hopes to develop novel modalities to interfere with pathological conditions which were long considered an inevitable decline in immune cell functions and normal aging. Immunometabolism in essence commonly describes the critical role of cellular metabolism in meeting the energy demands of circulating immune cells to counteract pathogens. More challenging is the conceptual and mechanistic understanding of the immunometabolic consequences viral infections have on tissue-resident immune cells. This will impact how we make inroads to comprehend the multifactorial events that facilitate hepatic viral persistence, end-stage liver diseases, and hepatocellular carcinoma (HCC). Data connecting dysregulated lipid metabolism and cellular damage in HBV and HCV-infected liver have been described.[1–4] An emerging paradigm in other chronic viral infections such as HIV purports that a metabolic shift from oxidative phosphorylation to glycolysis in immune cells perpetuates an inflammatory milieu that drives diseases progression in antiretroviral-treated and untreated individuals.[5,6] Interestingly, a similar metabolic signature of elevated glycolytic metabolism has long been recognized in cancerous cells.[7]

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Far less is known about the metabolic signatures of key immune cells such as Kupffer and cytotoxic CD8+ T-cells within the liver environment in HBV-infected people. However, CD8+ T-cells isolated from antigenic tumors have impaired functions, which may be attributed to competition by cancer cells for glucose in the tumor microenvironment.[8] This editorial will bring together recent evidence describing how liver injury and HCC may be accelerated in patients with HBV infection, and which metabolic pathways are being identified as potential targets to counteract or treat end-stage liver diseases and cancer. We will also present evidence that the occurrence of dysregulated glucose metabolism extends beyond HIV and hepatitis viral infection, hinted by Zika virus infection.

Using metabolites as novel diagnostic and prognostic markers of HBV disease progression

Serum alanine aminotransferase and HBeAg levels are universally used to track the natural course of HBV infection. However, work by Schoeman et al.[9] demonstrated that metabolomic analysis of serum metabolites may represent viable tools to monitor hepatic injury imposed by HBV infection. The team showed that while metabolite levels associated with amino acid influx and triglyceride species varied depending on disease progression, those indicative of oxidative stress steadily increased over the course of infection.[9] This study highlighted the potential utility of metabolic intermediates as diagnostic and prognostic tools to improve monitoring of people chronically infected with HBV.

Formal proof that dysregulated lipid metabolism contributes to tissue damage and HBV-induced liver diseases is lacking, but studies using an HBV infection cell model showed that lipid metabolites such as N-acetylaspartate and choline were reduced in HepG2 cells.[10] The pathophysiological role of these two metabolites in liver disease progression is unknown but this work supports previous findings that hepatic lipid metabolic dysregulation is a signature feature of HBV infection.[11,12]

Metabolic signatures of HBV-induced liver diseases

A recent study exploring the connection between the levels of metabolic intermediates in serum and HCC demonstrated that metabolites such as succinic acid, oxoproline, L-glutamic acid, and ornithine, belonging to unrelated pathways were significantly elevated in patients with HCC, suggesting that multiple signaling events are impaired.[13]

An elegant study by Li et al.[14] systematically dissects the cellular metabolic responses to HBV replication. Using an in vitro HBV infection model, they identified metabolic signatures associated with HBV replication. The team provided new evidence that both the hexosamine and the phosphatidylcholine biosynthesis pathways are disrupted by HBV replication. Indeed, HBV infection dysregulated glycolytic enzymes and increased secretion of lactate by HepG2 cells, a pattern typifying glycolytic metabolism and a characteristic feature of tumor cells. Additionally, HBV infection activated the pentose phosphate pathway leading the authors to speculate that the metabolic reprogramming also provides substrates for subsequent nucleotide synthesis used for HBV replication. Further emphasizing the importance of lipid metabolism in fatty liver disease in chronically infected HBV patients, Liu et al.[15] showed that total fatty acids were increased in HepG2 cells—a possible link to fatty liver diseases.

A more recent study by Yue et al.[10] employed a NMR-based metabolomics approach to investigate the effects of HBx on cell metabolism. They showed that genes involved in DNA damage were differentially expressed in HBx-expressing HepG2 cells which they interpreted as an additional mechanism by which HBV hijacks the cellular machinery to promote HCC. Taken together, the emerging paradigm that dysregulated glucose metabolism is a key mechanism that drives liver injury during chronic hepatitis viral infection represents a shift from the long-held lipocentric model. In this regard, we provided a model by which HBV infection disrupts key metabolic pathways in hepatocytes leading to liver diseases (Figure 1).

Glut1 is a key target of dysregulated metabolism in HBV infection

Glut1 is a key regulator of glucose influx and is controlled by the PI3K/Akt/mTOR pathway, an intracellular conduit that is dysregulated by HBx in rat hepatocytes.[16] It is tempting to implicate a key role for Glut1 in fueling the rapid cell division and growth that characterizes HCC, especially as Glut1 facilitates glucose uptake and glycolytic metabolism—a signature feature of cancer cells. However, Zhu et al.[17] showed that HBx upregulates expression of Alpha-fetoprotein, an oncogenic peptide which stimulates the PI3K/Akt/mTOR signaling pathway in HCC. Furthermore, Rawat and Bouchard[16] demonstrated that HBV replication in primary rat hepatocytes was significantly reduced when cells were treated with a nonspecific PI3K/Akt inhibitor LY294002. Official evidence to support direct involvement of Glut1 in HBV-induced HCC is unavailable; however, a comprehensive study by Lamontagne et al.[18] revealed that when primary rat

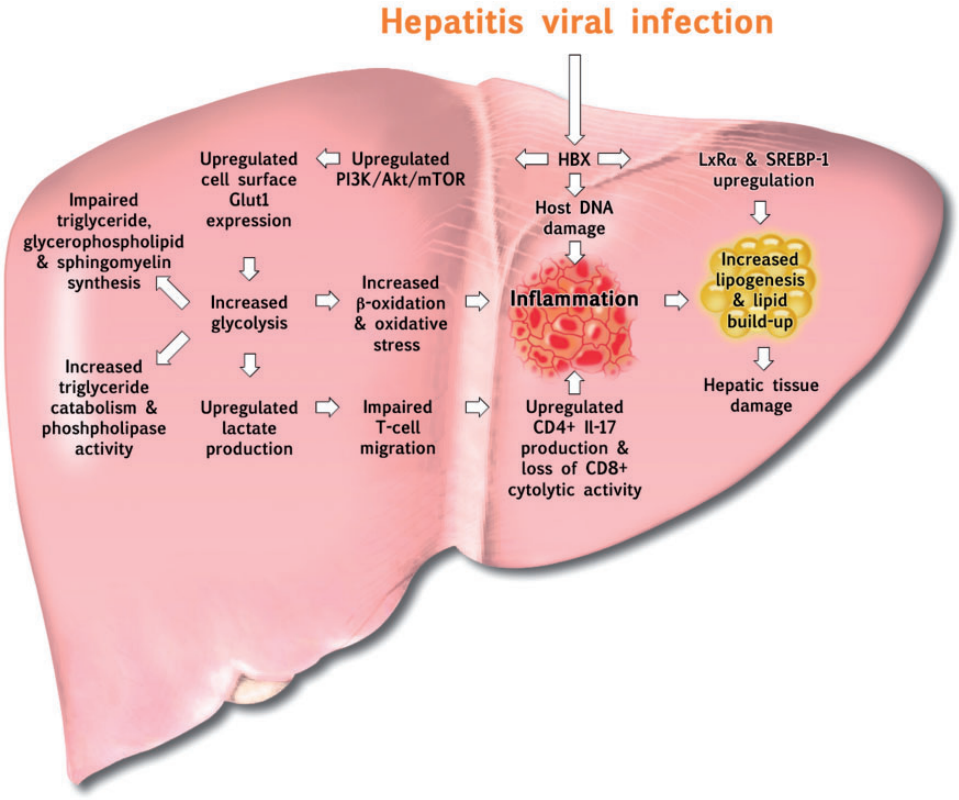

Figure 1. A model proposing mechanisms by which hepatitis B virus dysregulates glucose and lipid metabolism in infected liver. Hepatitis B virus X protein (HBx) causes metabolic reprogramming and cellular damage. Abnormal lipid accumulation and increased glucose uptake by cancer cells may drive end-stage liver diseases and HCC.
HCC: hepatocellular carcinoma.

hepatocytes were infected with an adenoviral vector expressing portions of the HBV genome, glycolysis was significantly increased, coinciding with a greater than twofold upregulation of Glut1 despite downregulation of Glut2. Metabolic profile analysis will continue to provide new information for novel antiviral modalities and strategies to delay progression of liver diseases in HCC and HBV-infected patients.

Immunometabolic regulations in other viruses

In general, most oncogenic viruses promote a cancer-inducing phenotype not only via direct insertion of viral DNA into the host genome but by also hijacking cellular physiology and metabolism.[19] Viral infection and cancers have similar metabolic demands requiring increased nutrient availability for immune cell function and for growth, metastasis, and proliferation of cancer cells. Metabolic modulatory effects have been shown in other oncogenic viruses such as human papillomavirus, HCV, and Epstein–Barr virus. Adenoviruses and human cytomegalovirus (CMV) also induce oncomodulatory effects but are not themselves directly oncogenic.[20]

Immunometabolic dysregulation has also been observed in exhausted HBV stimulated T-cells, which show an impaired capacity to utilize mitochondrial energy supply (OXPHOS), resulting in a dependence on glycolysis. This effect has been observed in CD8+ T-cells exposed to HLA-A2-restricted peptides derived from HBV, where Glut1 and IFNγ expression were significantly increased above the levels expressed by untreated CD8+ cells from human peripheral blood mononuclear cells.[21] Furthermore, Glut1-positive cell proportions developed at a greater rate for HBV HLA-A2-inoculated CD8+ T-cells than cells inoculated with nononcogenic CMV HLA-A2, positively correlating with expression of coinhibitory exhaustion biomarker PD-1, and suggesting early development of a cellular exhaustion phenotype brought about by upregulated glucose influx.[21]

Over the last few years evolving evidence indicates that glucose metabolic dysregulation in immune cells is a characteristic feature of HIV infection. It has been proposed that CD4+ T-cells increase Glut1 and glucose uptake to cope with higher energy demands during HIV infection.[5] In a fascinating study by Freemerman et al.,[22] Glut1 overexpression in a murine macrophage

cell line promoted excessive glucose uptake and metabolism and was associated with increased pro-inflammatory cytokine production via a reactive oxygen species pathway.[22] This and other studies support an evolving theory that overreactive glucose metabolism in immune cells plays a key role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis, obesity, cardiovascular diseases, and diabetes.

Glut1 upregulation may be linked to other pathological events independent of its functional role on immune cells. Recently, Blonz[23] theorized that Zika virus causes microcephaly by disturbing the placental Glut1 expression which limits access to glucose needed for normal fetal growth. This view is supported by observations that a genetic Glut1 deficiency is associated with an increased risk of acquired microcephaly in infants.[24] This unsubstantiated link between Glut1 and Zika-induced microcephaly is premature but warrants thoughtful consideration. In addition, the Zika virus itself could directly impair Glut1 levels and glucose uptake by immature brain cells. Zika infection may also cause a highly inflammatory response mediated by overmetabolically active placental resident macrophages or brain microglial cells.

Taken together, the metabolic machineries of host cells represent viable targets for novel treatments to improve immune responses and treat cancers and inflammatory diseases associated with viral infections. For example, Glut1 inhibitors have already been developed for anticancer therapy and include drugs such as fasentin[20] and 4-[[[[4-(1,1-dimethylethyl) phenyl]sulfonyl]amino]methyl]-n-3-pyridinylbenzamide (STF-31).[25]

Targeting metabolism to prevent HBV and HCV-related cancer development

Both HBV and HCV infection activate glucose metabolic machineries including the PI3K/Akt/mTOR and EGFR/RAS/MAPK pathways.[21] These pathways present potential targets in HCC treatment and are already being explored. In HCC cell lines mTOR blockage by rapamycin or everolimus treatment inhibited tumor growth.[26] Targets in EGFR/RAS/MAPK pathways are also being exploited and include the use of monoclonal antibodies (mAbs) cetuximab and trastuzumab, which are raised against EGFR and ErbB2/Her2/neu, respectively. These mAbs are already approved for treatment of various cancers.

A recent study by Bard-Chapeau et al.[27] elegantly showed that many of the genes linked to tumorigenesis in a murine model of HBV-induced HCC were linked to aerobic glycolysis. HBV-infected liver transplant recipient patients with HCC had significantly reduced tumor size, decreased PI3K, AkT, AMPK, mTOR, 4EBP1, and S6K protein expression and phosphorylation when given a combination of rapamycin and a mitochondrial influencer, metformin.[28] The glycolytic effects of these genes could potentially be ameliorated by existing drugs including hexokinase inhibitors (2-deoxyglucose, 3-bromopyruvate) and glyceraldehyde-3-phosphate dehydrogenase inhibitors (iodoacetate, gossypol).

The PI3K/AkT/mTOR pathway has also been suggested to stimulate T-cells during adaptive immune response to HBV. He et al.[29] have shown that rapamycin inhibited the phosphorylation of TSC2, an upstream regulator of mTOR, stimulated IL-12 production in macrophages while simultaneously downregulating IL-10 production, and boosted TLR-induced antigen-specific T- and B-cell responses to HBV and HCV vaccines. As high IL-12 and low IL-10 production is vital to CD4+ T-cell stimulation and differentiation upon viral inoculation,[30–33] the collective literature suggests that the PI3K/Akt/mTOR pathway is a prime therapeutic target for HBV vaccination development.

Conclusion

As the immunometabolism field galvanizes momentum so will the interest of pharmaceutical companies to keep pace with basic research in this area. Equally important is the need for methodologies to investigate metabolic dysfunctions in small number of cells available from study participants. In this regard Palmer et al.[34] have comprehensively reviewed several important methods that can be applied to investigate cellular metabolic dysfunctions in disease settings.

Acknowledgements

The authors would like to thank Catherine Cherry for proofreading the manuscript, and Keryn Lyn Masson for her assistance and technical advice. The authors gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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