REVIEWS

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Modern views on the role of *X* gene of the hepatitis B virus (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) in the pathogenesis of the infection it causes

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The review presents information on the role of hepatitis B virus (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) (HBV) *X* gene and the protein it encodes (X protein) in the pathogenesis of viral hepatitis B. The evolution of HBV from primordial to the modern version of hepadnaviruses (*Hepadnaviridae*), is outlined as a process that began about 407 million years ago and continues to the present. The results of scientific works of foreign researchers on the variety of the influence of X protein on the infectious process and its role in the mechanisms of carcinogenesis are summarized. The differences in the effect of the X protein on the course of the disease in patients of different ethnic groups with regard to HBV genotypes are described. The significance of determining the genetic variability of *X* gene as a fundamental characteristic of the virus that has significance for the assessment of risks of hepatocellular carcinoma (HCC) spread among the population of the Russian Federation is discussed.

Key words: *hepatitis B virus (HBV); gene X; protein X; carcinogenesis; hepatocellular carcinoma (HCC); review*

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НАУЧНЫЙ ОБЗОР

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Современные представления о роли гена *X* вируса гепатита B (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) в патогенезе инфекции, вызванной вирусом гепатита B

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В обзоре представлена информация о роли *X* гена вируса гепатита B (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) (ВГВ) и кодируемого им белка X в патогенезе вирусного гепатита B (ГВ). Рассмотрена эволюция возбудителя от первоосновы до современного варианта гепаднавирусов (*Hepadnaviridae*) как процесс, начавшийся около 407 млн лет назад и продолжающийся до настоящего времени. Обобщены результаты научных трудов зарубежных исследователей о многообразии воздействия белка X на течение инфекционного процесса и роли этой вирусной структуры в механизмах канцерогенеза. Описаны различия в характере влияния белка на течение заболевания у пациентов различных этнических групп с учётом генотипической принадлежности ВГВ. Обсуждается значение определения генетической вариабельности гена *X* как фундаментальной характеристики вируса, имеющей значение для оценки рисков распространения гепатоцеллюлярной карциномы (ГЦК) среди населения Российской Федерации.

Ключевые слова: *вирус гепатита B (ВГВ); ген X; белок X; канцерогенез; гепатоцеллюлярная карцинома (ГЦК); обзор*

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Introduction

Viral hepatitis B (HBV) remains a major global public health problem, affecting the health of the population in many countries and causing a large number of deaths directly related to HBV as well as from HBV-related liver cirrhosis (LC) and hepatocellular carcinoma (HCC). The latter is one of the most serious complications of HBV infection [1]. According to estimates from the World Health Organization (WHO), HBV is responsible for 820,000 deaths annually, including more than 650,000 deaths from LC or HCC and around 130,000 deaths from acute HBV [1, 3]. Currently, 296 million people are living with chronic HBV infection, and another 1.5 million new infection cases are reported annually. HCC is the third leading cause of death from cancer worldwide [2]. At present, after the onset of HCC, the median survival time does not exceed 11 months, and the 5-year survival rate is as low as 6.9% [3].

Considering highly unfavorable prognoses and high prevalence of both diseases in many countries, the development of liver cancer in patients with chronic hepatitis B (CHB) infection has attracted a lot of attention from researchers. Many recent studies have demonstrated that HBV-induced carcinogenesis has a strong relationship with properties and interactions

of proteins encoded by virus genes, primarily, with the interaction between the X protein, target genes, and proteins of the host cell. Assessment of the role of the *X* gene and the namesake protein encoded by it during the pathological process initiated by HBV is a focus area of studies performed in different countries [9–17]. The presently known amino acid substitutions in the X protein trigger new effects of the virus on hepatocytes, which are not characteristic of its wild-type variant, such as carcinogenesis initiation, genome instability, generation of cancer stem cells, enhanced replication of the human immunodeficiency virus (*Retroviridae; Orthoretrovirinae; Lentivirus: Human immunodeficiency virus*) (HIV), carcinogenesis progression in patients with mixed CHB and chronic viral hepatitis C (CHC) as well as superinfection with the hepatitis delta (D) virus (HDV) [9, 10, 13, 16–18].

The aim of this review is to present the up-to-date information and summarized results of the studies addressing the assessment of the role of the HVB *X* gene and the encoded protein in HBV pathogenesis.

Evolution of the hepatitis B virus

The fundamental studies on the HBV origin have produced quite interesting results, which give insight into many properties of the virus. Its archeological age was estimated when this pathogen had been isolated from 400-year-old mummified remains discovered in Korea and Italy [4]. The HBV genome was also sequenced from 7-thousand-year-old skeletal remains in Eurasia [4]. The screening identified viruses of the family *Hepadnaviridae* in a number of different representatives of the animal world, including capuchin monkeys (*Cebus*) in South America, blue bream (zope) (*Ballerus ballerus*) in North America, flat needlefish (*Ablennes hians*) in the Eastern Sea, Australian bass (barramundi) (*Lates calcarifer*), Tibetan frogs (*Nanorana parkeri*), African cichlids (*Cichlidae*), and in 2 species of lizards (*Lacertilia*) [4].

The identification of hepadnaviruses integrated into a germline of some birds (*Aves*) and reptiles (*Reptilia*) was performed by Suh A. et al. and demonstrated an ancient origin of this family, the prototype of which was formed more than 200 million years ago, i.e. in the Triassic period of the Mesozoic era [21, 22]. The integration of viral DNA into the genome of the avian germline, insertion of the endogenous viral element (eHBV), which indicated that the species barrier had been crossed into birds, took place 77–90 million years ago (the Cretaceous period of the Mesozoic era). The evolutionary model built by Revill P. et al. [4] suggests that structural predecessors of the future hepadnavirus could be retroelements of genomes in insects (*Insecta*), which formed the basis for the HBV polymerase gene [5]. Throughout the evolutionary process, this virus acquired a core gene, as demonstrated by the study of Lauber C. et al. [38]. In the opinion of the authors of the study based on the phylogenetic analysis and the estimated date of the division of hepadnavirus of fish (*Pisces*) (the common ancestor) into hepadnavirus of ray-finned fish (*Actinopterygii*) and lobe-finned fish (*Sarcopterygii*), the evolution of the hepadnavirus began more than 437 million years ago, or in the Ordovician period of the Paleozoic era. The *S* gene, which is also responsible for hepatotropism of the virus, was formed throughout its evolutionary development in birds [23, 24]. According to the data from Suh et A. et. [21, 22] and van Hemert F.G. et al. [12], when crossing the species barrier (from birds to mammals (*Mammalia*)) 25–10 thousand years ago, the hepadnavirus acquired a new *X* gene encoding the respective protein.

It should be noted that this gene, most likely, owes its regulatory functions to its origin. There are 2 theories of the *X* gene emergence. One of these is described in the work by van Hemert F.J. et al. [12], who found that the X protein was similar to the human thymine-DNA glycosylase (TDG) enzyme, a key participant of the excision repair of nucleotides. The authors assumed that the *X* gene emerged when during the evolution the hepadnavirus "captured" the respective sequence from the host genome approximately 10 thousand years ago. According to the researchers, the X protein inhibition of the TDG initiated excision repair of nucleotides of cellular DNA can be associated with the origin of this protein from the above cell enzyme. The other theory of the *X* gene emergence was offered by Suh A. et al. [21, 22]. They assumed that the X-like open reading frame (ORF) could be formed during the process of evolution through a segmental duplication of precore/core ORF and a subsequent genome overlapping. Their assumption is based on gene and precore/core ORF overlapping in the genome of viruses belonging to the genus *Avihepadnaviridae*.

The long evolution of HBV played a crucial role in its broad representation in the animal world. The fundamental difference between *Orthohepadnaviridae* and *Avihepadnaviridae* is that the latter do not have a fully functional *X* gene; the fact that in natural environment, HCC has been recorded only in mammals is associated with the presence of the *X* gene and the activity of the encoded protein [16]. In the meantime, in 2001, Chang S.F. et al. [16] reported about X-like ORF detected in the HBV genome of ducks (*Anatinae*), which let them assume that HBV is still going through evolution. The study of the origin and evolutionary development of this virus shed light on its characteristics, including high prevalence in different species of animals, emergence of new recombinants, existence in the body as a quasi-species population, and involvement of multiple host organs, and also helped researchers understand why the pathogen is resistant to antiviral therapy.

The structure of the *X* gene of hepatitis B virus

In the HBV genome, the *X* gene region extends from nt 1374 to 1838 [17, 19, 20]. At the same time, in the opinion of González A. et al., the region of this gene should also include the transcription initiation site covering a non-coding sequence in the region from nt 1255 to 1374 [19]. An important feature of the HBV genome is its compact organization. The *X* gene partially overlaps the polymerase gene at nt 1374–1621 and the precore/core gene at nt 1814–1838 [17, 19, 20]. The sche-

matic organization of the *X* gene (nt 1374–1838), which also includes its transcription initiation site (nt 1171–1361), is shown in **Figure** [20, 21].

The *X* gene transcription initiation site (non-coding region) as well as its coding region (nt 1374–1838) contain hyper-conserved regions located at positions from nt 1255 to 1286 and from nt 1563 to 1602 [19]. The overlapping sequences of polymerase genes, precore/core gene, and multiple regulatory non-coding sequences critical for replication and transcription of the virus genome make this part of the gene a potentially significant target for therapy [19].

Structure and functions of the hepatitis B virus X protein

The protein translatable by the *X* gene consists of 154 aa; its molecular weight is 17 kDa, and it stimulates transcriptional transactivation of a variety of cellular and viral promoters. The X protein includes 2 domains: the N-terminal domain encoded by 5'-region of the *X* gene from nt 1374 to 1523 (1–50 aa), and the C-terminal domain encoded by 3'-region from nt 1524 to 1838 (51–155 aa). The first of them mediates the pro-apoptotic function, while the C-terminal domain is responsible for transactivation processes [20]. Lately, it has been found that the N-terminal domain of the protein is more variable compared to the C-terminal domain, as the latter contains 3 of 4 conserved amino acid residues [19, 40, 49]. Note that the variability of the domains depends on the HBV genotype. The N-terminal domain has more variable amino acid positions (6, 12, 26, 30, 38, 40, 42) than the C-terminal domain (78, 91, 101, 102, 118, 119) [49]. The region of the more conserved C-terminal domain of the protein also has the Kunitz-like domain located at 2 separate positions – from 61 to 69 and from 131 to 142 aa. It is able to inhibit functions of some cellular proteases [19]. Prieto C. et al. [72] found that the X protein owes its dimerization to its region rich in serine (Ser, S) and proline (Pro, P).

The X protein is known to have a highly pleiotropic nature; its impact on the infection process is quite versatile. Sung W.-K. et al. [11] identified 184 target genes and 144 transcription factors that can participate in interaction with this protein, though only some interactions have been studied so far. The pleiotropic effect of the X protein depends on its localization (in the nucleus of cells or subcellular localization), which affects its interaction with nuclear and cytoplasmic factors [42, 43]. Based on the data from Bouchard M.J. et al. [13] and Kornyeyev D. et al. [43], this protein being localized in the nucleus and cytoskeleton can exist for around 3 hours; in the cytosol – from 15 to 20 min. The results of the studies performed by Belloni L. et al. [42] demonstrate that nuclear localization of the X protein results in its activation of cellular proto-oncogenes. In their turn, in the review, Ali A. et al. [44] state the fact that the nuclear localization of the protein is associated with the insertion at position 204 of the nucleotide sequence. In the nuclear localization, the X protein participates in regulation of the transcription of the virus and cell genomes and exerts its oncogenic potential, regulating the expression of host genes, interacting with components of the basal transcription machinery (RPB5, TFIIB, TBP) and with specific transcription factors [44].

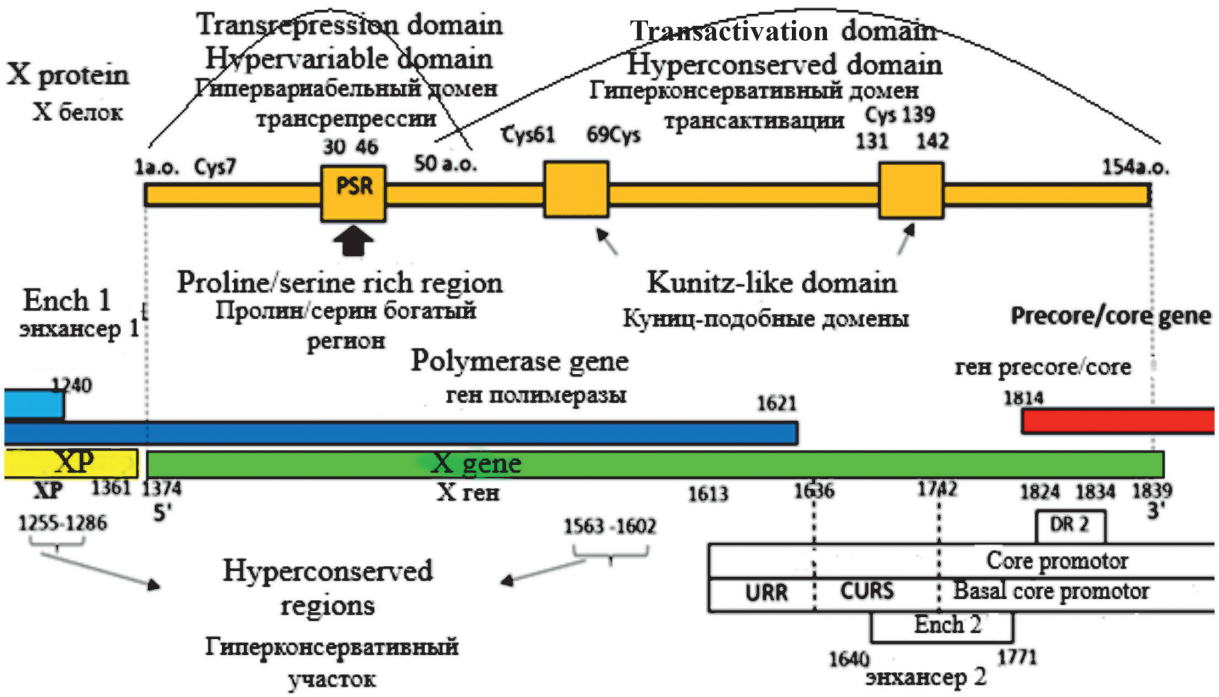

Fig. The structure of *X* gene and translated X protein of the hepatitis B virus.
Рис. Строение гена *X* и транслируемого им белка X вируса гепатита B.

When localized in the cytoplasm, this protein is associated with the mitochondrial outer membrane, changing the conductance of the anion membrane channel, thus causing modulation of apoptosis [13, 42, 43].

The primary function of the X protein – regulation of the expression of virus genes and cell genome – is performed through several ways: destruction of the chromosome Smc5/6 structural maintenance complex (in the hepatocyte it performs the function of virus factor restriction), the SETDB1 chromatin modifier preventing the transcription of the virus genome; operation of cellular epigenetic mechanisms; activation of cellular proto-oncogenes [42, 45, 48]. There is an opinion that the protein acquired its ability to destroy Smc5/6 while crossing the species barrier from birds to mammals; this ability may be seen as the primary function of this protein [46, 47]. Belloni L. et al. [42] found that the interaction between the X protein and acetyltransferases (CBP, p300, PCAF) results in acetylation of cell and virus genomes; it also causes activation of cellular proto-oncogenes and transcription of the covalently closed circular DNA (cccDNA) of the virus. The X protein-mediated hypermethylation of promoters of cellular tumor gene suppressors is implemented through enzymes – DNA methyltransferases DNMT1, DNMT3A1, and DNMT3A2 activated by this protein, leading to inhibited transcription of a variety of tumor suppressors in the cell.

The X protein can also activate transcription of cellular proto-oncogenes and the virus genome, interacting with components of the core transcription complex of RNA polymerase II (RPB5, TFIIB, TFIIH), and specific transcription factors (ATF/CREB, ATF3, c/EBP, NF-IL-6, Ets, Egr, SMAD4, Oct1, RXR-receptor, p53).

It has been found that phosphorylation of Ser at positions 155, 162, and 170 of the core protein is triggered with participation of the X protein and is essential for stepwise encapsidation of the virus genome, resulting in the semi-circular DNA capable of further replication. Thus, it has been found what role this protein plays in replication of the HBV genome [71].

Mechanisms of viral hepatitis-induced carcinogenesis associated with X protein functions

It has been proven that the X protein and HCC have a pathogenic relationship, which is brought into effect through different ways: through integration of the virus genome into the cellular genome, through activation of proto-oncogenes, and hypermethylation of tumor suppressor genes [42, 45, 48]. In 2006, researchers discovered another direct oncogenic effect when some HBV genome proteins initiate generation of cancer stem cells (CSC) [62].

Su F. et al. [50] describes the carcinogenesis mechanism associated with the pro-apoptotic activity of the X protein. The researchers assume that the initiation of oncogenic activity can involve not only the X protein characterized by enhanced transformation potential backed up by its mutations, but also altered variants of this protein, which demonstrate inhibited transformation activity due to activation of the pro-apoptotic domain [13, 50]. In the authors' opinion, variations of altered proteins co-exist as quasi-species [13, 18, 50]. The experiments demonstrated that the X protein pro-apoptotic activity, which comes into effect during inhibition of the C-terminal domain (responsible for transformation activity and interaction between the protein and the tumor necrosis factor alpha (TNF-α)), induces the activation of apoptosis and natural survival of neoplastic hepatocytes. The latter, in their turn, synthesize intensively mitogenic growth factors. The pro-apoptotic function of the X protein of the wild-type virus contributes to spread of the virus within the affected liver. The apoptosis of hepatocytes results in a sharp increase in hepatocyte growth factors, which are indispensable for regeneration of the organ, creating conditions for HBV dissemination in new non-infected hepatocytes [50].

One of the main aspects of the oncogenic effect is associated with the HBV ability to integrate into the genome of the host cell [44, 46]. Shafritz D.A., Kew M.C. [52] were among the first researchers to describe the relationship between this ability and malignant cellular transformation. The subsequent studies performed by different research groups helped identify several carcinogenesis-mediating effects associated with HBV integration. Some of them are cis-mediated insertion mutagenesis of virus genes and the host cell; induction of chromosome instability due to the integrated DNA; expression of mutant HBV genes from the stable integrated form [51, 53, 54]. The studies have demonstrated that the integration of the virus genome into the cellular genome takes place in the early stage of infection. It was experimentally confirmed that the first merger of both genomes takes place within the first 30 min after infection. The comparison of the frequency of integration into HCC affected and healthy hepatocytes showed that in tumor tissue, integration is more typical of coding genomic sites, while intact cells are characterized by integration into introns. The altered X protein increases the number of sites and the frequency of integration events by activating the signaling pathways leading to genome damage.

Integration into coding regions results in the expression of chimeric oncogenic proteins; it has been found that the expression of cellular genes with inserted HBV fragments increases regardless of the localization of insertion sequences. The integration of the virus into the cell genome of tumor tissue (in contrast to healthy hepatocytes) takes place in repetitive genomic regions, resulting in transcription activity of proto-oncogenes. The conducted studies showed that the frequency of integration events correlated with HBV progression and its severe form as well as with HCC development at a younger age, bypassing the cirrhotic stage. In addition, it was found that such integration was observed both in chronic and acute HBV, regardless of the patient's age [51, 53, 54].

The integration of the virus genome is accompanied by its fragmentation; as a result, the *X* and *S* genes can be expressed, which are close to their promoters, while *core* and *Pol* genes, being distanced from their promoters, cannot be expressed [51, 53, 54]. It has been found that the *X* gene integration causes gene fragmentation and

multiple breaks in the nucleotide sequence. Retrotransposons SINE, THE-1B-LTR, MER-5B, and LINE-2 are initial sites of HBV integration into the human genome. Integration into retrotransposons can result in spread of viral insertions throughout the genome and the subsequent development of HCC. It was proved that genomic fragments of the virus can be integrated into proto-oncogenes: NBPF-1, PRR-16, PRKG-1, RunX1, hAT-18, DNTNP, PEB-4. FAM90, PCDH-15, lncRNA, and C14Orf29 as well as genes encoding the human telomerase reverse transcriptase (hTERT), the mixed-lineage leukemia 4 (MLL4) and the gene encoding cyclin E1 (CCNE1) (CCNE1) [51, 53, 54].

CSC is an important factor of the HBV-associated carcinogenesis. A number of experiments demonstrated the ability of viral proteins to induce generation of such cells, which would be capable of self-renewal and differentiation into all of the cancer cell lineages present within the tumor [59–61]. It was found that the X protein can activate mitogenic signaling cascades via transcription factors NF-κB, AP-1, AP-2, c-EBP, and ATF/CREB, thus facilitating the generation of CSC. During the same experiments, the authors showed that the integration of the *X* gene and the generation of the X protein with the carboxyl terminus deletion initiate the transcription factors of pluripotent stem cells of Oct4, Nanog, Klf4 lineages – potential precursors of CSC, as well as the markers of the latter, such as EpCAM and β-catenin. Having lost the COOH-terminus, the X protein is able to initiate generation of a subset of CD133 markers of the above cells [66–68]. CD133, which is also known as AC133 or prominin-1, is a CSC surface antigen; in practical medicine, it serves as a diagnostic marker indicating the presence of CSC in a patient and a high probability of a tumorigenic process in some localizations. It is also extracted from tumors of different organs, including the brain, colon, pancreas, prostate, lungs, and liver [83]. The positive test for CD133 indicates that the patient has CSC and is of great importance in the diagnosis of HCC [59–63]. The HCC studies demonstrate that the cellular elements expressing CD133 have a higher proliferative potential than the cells that do not have this marker [62, 63]. In addition, it has been found that the CD133 presence contributes to cancer cells resistance to chemical substances, radiation, apoptosis, and to more intensive chemo- and radioresistance of the tumor. According to the data from Li Z. [63], the cytoplasmic CD133 expression correlates with tumor progression, while the nuclear one is associated with more gradual development of HCC.

Association of nucleotide substitutions in the *X* gene and amino acid substitutions in the X protein with carcinogenesis mechanisms in viral hepatitis B

Significant attention has been given to the impact of substitutions in the *X* gene and the encoded protein on the oncogenic potential of HBV. The recent studies confirm the significant role of single nucleotide/amino acid substitutions and their combinations that can produce a synergetic effect. It results in faster progression of the pathologic process in HBV to LC and HCC. The authors pointed out the variability of scenarios for HBV infection in patients from different ethnic groups [8, 11, 16].

Currently, 2 dual substitutions 1762T/1764A and 1764T/1766G as well as the single substitution 1758C have been most extensively studied [20]. As it has been demonstrated by Kim H. et al. [20], the presence of dual substitution 1762T/1764A in the *X* gene results in 3 nonsynonymous nucleotide substitutions: G1386A/C, C1653T, T1753V and, consequently, in amino acid substitutions V5M/L, H94Y, I127V in the X protein. Furthermore, dual substitution 1762T/1764A is frequently combined with the deletion in the region of the C-terminal domain of this protein. The above dual substitution, in the opinion of many researchers, is not associated with any ethnic group and is common among patients with HCC [8, 10–13, 16, 17, 35].

The detection of amino acid substitution F30V by Salpini R. et al. [15] in the highly conserved region of the N-terminal domain of the X protein is of special interest. The authors explain its presence by the suppressed apoptosis of damaged cellular elements. As a result, damaged cells tend to remain and accumulate in the genome, contributing to development of HCC. Nevertheless, the above substitution is not associated with the progression of the cell cycle. According to researcher, the malignant transformation and the subsequent development of HCC in HBV caused by the virus with the altered X protein take effect through two independent ways which can operate concurrently, as in the infected body, HBV generally exists as different genetic variations or, as described above, as quasi-species. Thus, one pathway of carcinogenesis, which is associated with the X protein with activated transformation domain, facilitates cellular tumor transformation, while the other pathway associated with the same pro-apoptotically active protein promotes the selection of neoplastic cells producing mitogenic growth factors [5, 15, 44].

At present, the scientific community increasingly reports that HBV can initiate a tumorigenic process not only in liver tissue, but also in other organs such as pancreas, stomach, oral mucosa, and colon. It should be noted that carcinogenesis of extrahepatic localization, in the opinion of some researchers, is associated with the effect produced by the X protein on the transcription of proto-oncogenes of the human cell [55–58]. The studies addressing HBV-associated neoplasms are generally focused on such diseases as gastric cancer and non-Hodgkin lymphoma. The participation of HBV in development of gastric cancer, in the opinion of Niedźwiedzka-Rystwej P. et al. [57], can be proved by a significantly higher amount of the X protein in tumor tissue as compared to healthy tissue.

Special attention should be given to the study by Kim H. et al. [67] who reported about the detected deletion in the X protein at positions from 127 to 134 aa; the deletion was associated with its decreased secretion and delayed virion assembly. The researchers assume that this genetic alteration can cause development of asymptomatic HBV infection in vaccinated individuals. A number of studies have identified the relationship between the severity of

the disease/its outcome and the genotypic profile of the virus; note that ethnicity of patients is also seen as an important factor. For example, the examination of a cohort of patients in North India showed that infection with the HBV A genotype most frequently results in chronic HBV, while the similar Spanish group of examined individuals infected with the same genotype demonstrated high frequency of the spontaneous viral clearance [5, 6, 66]. In addition, there are data on "ethnic" specificity of some substitutions in the *X* gene and the respective HBV protein. According to Tuteja A. et al. [6], for the population of North India, the following amino acid substitutions: V37L, L98C, E126R, V133Y, A144H, P145Q were singled out as specific for the X protein. Based on the results of another study conducted in India, researchers assigned other 5 substitutions of amino acids: L37I, S46P, H86P/R, L98I, T105A to specific [70]. Specific nucleotide substitutions were also found in HCC patients examined in China and Japan; 6 substitutions of nucleotides *X* gene (1485T, 1653T, 1470A, 1479A, 1575G, 1719G) were found to be ethnic-specific for HCC [64]. In the review by Li W. et al. [68] addressing the analysis of genetic variability of this gene in CHB patients from Japan and China, 5 specific, in the authors' opinion, nucleotide substitutions: 1383C, 1485T, 1631T, 1719T, and 1800C were pointed out.

The report of Tuteja A. et al. [6] about the detected inter-genotypic recombinant HBV A/D is worthy of attention; in this recombinant, the *X* gene was associated with the D genotype, while the remaining genome was associated with the A genotype. The researchers think that such genetic recombination of HBV contributes to higher carcinogenicity and reduces the efficiency of administered antiviral drugs.

Special attention should be paid to difficulties encountered by researchers studying the impact of individual substitutions in the nucleotide sequence of the *X* gene. They are caused by the existence of overlapping sequences in this gene and basal (main, minimum) core promotor (BCP). The exploration of genetic variability of the *X* gene requires genetic dissection, as substitutions in the overlapped sequence result in mutations of regions in this gene and BCP [65]. The latter represents a regulatory sequence of the virus genome, which initiates transcription of precore and pregenomic RNA (pgRNA). The dissection BCP and the *X* gene was performed to assess the impact of the substitution in the *X* gene on the pgRNA synthesis and consequently, on the replication of the HBV genome. It was found that the increased replication of the virus genome was caused by changes in BCP rather than in the *X* gene [65].

The summarized results of the search of information about nucleotide substitutions in the *X* gene and amino acid substitutions in the X gene-encoded protein associated with HCC development are presented in **Table**.

The effect of the X protein on the course and outcome of viral hepatitis B in delta infection and mixed infection with human immunodeficiency virus

The evolutionary analysis of the infection process caused by HBV and HDV showed an expected reduction in DNA replication of the first of them, both in coinfection and superinfection [10]. The authors of the study performed on a group of patients in Spain explain the effect by the interaction between the cellular RNA polymerase II, which is required for HBV genome replication and the HVD large HDAg antigen (L-HDAg), and by the activation of the cellular antiviral protein MxA. L-HDAg interacting with HBV enhancer 2 induces nucleotide substitutions in the *X* gene, which translating to the X protein, inhibit the replication of the HBV genome. Seeing it as a common pattern, researchers point out that delta infection is associated with an increased total number of nucleotide substitutions in the HBV genome. Note that in viral hepatitis D, nucleotide substitutions are localized in the region nt 1255–1611, partially including the site of *X* gene transcription initiation and the respective gene. According to the authors, the higher genetic variability of the above HBV genomic region is associated with the effect of innate immunity and/or with interaction of L-HDAg with RNA polymerase II. The study performed by Goto T. et al. [73] who were also exploring the interaction between HVD L-HDAg and the X protein, demonstrated the activation of cellular proteins: transforming growth factor beta (TGF-β) and activating protein 1 (AP-1) (transcription factor). These proteins perform the function of regulators of proliferation, cellular differentiation and other processes by binding to SMAD3 and STAT3 proteins and initiating the c-Jun signaling pathway, which activates the latent form of TGF-β and directly phosphorylates SMAD3. The combination of these processes results in strengthening of translation profibrotic molecules. The above interactions, as seen by the authors of the study, trigger the development of liver fibrosis in patients. In addition, the interaction between HVD L-HDAg and the X protein activates the transcription SRF factor, which is an important promoter and regulatory element involved in increased production of growth factors and proteins of proto-oncogenes.

The present-day epidemiological situation for HBV and HIV infection in the world is described as an epidemic with permanently increasing numbers of involved people. Having common transmission routes, both pathogens are frequently recorded as HBV + HIV mixed infections. Note that the reciprocal impact of these diseases, which includes the role of the X protein in changing the development of HIV infection, has been proven. As demonstrated by Gómez-Gonzalo M. et al. [18], the HBV/HIV mixed infection is accompanied by suppression of antiviral APOBEC3G proteins and subsequent activation of HIV RNA replication. The above effect is driven by the ability of the X protein to induce continuous replication of HIV-1 genome and transcription of long terminal repeats (LTRs) of RNA of this virus RNA through interaction with its Tat protein and T cell activation signals. The replication of the HIV-1 genome is activated by the X protein binding to the HIV-1 Sp1 LTRs site and by the interaction of HBV X and HIV-1 Tat proteins. The replication of the latter is also initiated by the X protein activation of NF-κB and NF-AT proteins. It has been found that its ability to activate HIV-1 RNA replication is associated

with substitutions in the C-terminal domain of the X protein, which are localized from 57 to 104 aa.

Conclusion

The review of scientific publications focusing on the impact of the *X* gene and the respective protein on the development of HBV infection shows that there are three main impact areas characterized by a variety of involved mechanisms. Firstly, the *X* gene is responsible for HBV integration into the genome of the host cell. Secondly, the X protein regulates HBV DNA transcription by employing direct and indirect mechanisms. Thirdly, this protein is the main factor initiating multiple carcinogenesis mechanisms. The analysis of the literature shows that researcher give considerable attention to the impact of different nucleotide substitutions in the *X* gene and corresponding amino acid substitutions in the namesake protein on the course of HBV infection and development of such complications as LC and HCC.

Most of the subject-related studies have been performed in such countries as China, Korea, Japan, India, thus

Table. Nucleotide substitutions in *X* gene and amino acid substitutions in X protein available in the publications
Таблица. Нуклеотидные замены в гене *X* и аминокислотные замены в белке X, описанные в литературе

Nucleotide substitutions in *X* gene Нуклеотидные замены в гене *X*	Amino acid substitutions in X protein Аминокислотные замены в белке X	Patient ethnicity Этническая принадлежность пациента	References Источник
1762 (T), 1764 (A)	K130M/V131I	Korea, Saudi Arabia, China Корея, Саудовская Аравия, Китай	[44]
G1386A/C	V5M/L	Korea, China Корея, Китай	[20]
C1653T	H94Y	Korea, China, Japan Корея, Китай, Япония	[20]
T1753V	I127V	China Китай	[20]
	X8Del	Korea Корея	[20]
	I127T/K130M/V131L	Saudi Arabia Саудовская Аравия	[13]
	K130M/V131I/ F132Y	Saudi Arabia Саудовская Аравия	[13]
G1727, C1741, C1761, T1773, T1773/G1775, T1673/G1679, A1757/T1764/G1766		Saudi Arabia Саудовская Аравия	[13]
	F30V	France, Italy Франция, Италия	[15]
1485T, 1653T, 1470A, 1479A, 1575G, 1719G		Japan Япония	[20]
T1674C/G, T1753V, T1768A, C1773T		China Китай	[20]
A1383C, C1485T, C1631T, G1719T, T1800C		Japan, China, Korea Япония, Китай, Корея	[20]
	V37L, L98C, E126R, V133Y, A144H, P145Q	Northern India Северная Индия	[6]
	L37I, S46P, H86P/R, L98I, T105A	Indonesia Индонезия	[70]
Insert 204AGGCCC in combination with G260A and G/C/T264A replacements Вставка 204AGGCCC в сочетании с заменами G260A и G/C/T264A		–	[44]
	Deletion of 14 amino acids in the COOH domain Делеция 14 аминокислот в COOH-домене	–	[44]

Note. «–», no data available.

Примечание. «–» – отсутствие данных.

demonstrating the high significance of the HBV problem for these countries. In Russia, data on genetic variability of individual HBV genes are almost non-existent, except for a few studies addressing the heterogeneity of the *S* gene and the encoded protein. Taking into account that patients' ethnicity, as shown by the above publications, is an important factor affecting the development of induced HBV infection and its complications, the related studies acquire an increased importance in Russia, which is a multinational and multiethnic country.

The findings on genetic variability of HBV are both of fundamental theoretical and practical importance. New information about the genetic diversity of HBV and the associated types of HBV infection is highly important for assessment and prediction of the general epidemiological situation as well as for evaluation of the efficiency of epidemic control measures in the target country. The identification of substitutions in virus genes can be used for developing a patient-specific approach. Note that scarcity of this information in Russian scientific and medical practice significantly limits the idea about the scale of problems associated with HBV and urges further research.

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