Introduction

In western countries breast cancer (BC) is one of the leading cause of cancer related death in women, whereas, its incidence is low in Chinese females. The studies of migrant populations have shown that when low-risk groups (e.g., Chinese groups) move to high-risk regions (Hawaii or mainland USA), their incidence of BC increases rapidly, approaching the rates of the host population within one or two generations (1,2). These marked differences in BC incidence suggest that environmental factors might have a major influence on the risk of developing BC; in fact, environmental factors might be more important than the influence of genetic factors, perhaps with the notable exception of the familial BC syndromes. So, what are the main prevalent and environmental carcinogenic factors to cause the early age onset of BC in China?

Several viruses have been implicated in the pathogenesis of a subset of BC (3). A population-based case-control study about association between chronic viral hepatitis infection and BC risk suggested that chronic hepatitis C virus (HCV) infection was associated with early-onset BC (4). Similarly, as a hepadnavirus, hepatitis B virus (HBV) infection is more common than HCV in China, where every surgical patient should undergo routine examination of HBV serological markers and liver function tests for peri-operative preparation before operation. Though with the nationwide vaccination program since 1992, the

Abstract: Lifestyle and family history are two of the most important risk factors for breast cancer (BC). However, these risk factors cannot explain the differences in the incidence and early BC onset among Chinese females compared to their western counterparts. We propose in this hypothesis the potential mechanism of indirect oncogenesis of hepatitis B virus (HBV) in causing BC through its persistence as occult infection and continuous replication with long term subtle liver damage. Estrogen is mainly deactivated in the liver and long term necro-inflammatory damage to liver may result in persistent high level of estrogen, which is a dominant risk factor for BC. HBV may also directly affect the breast cells through its cis and trans effects of HBx which may act as oncoprotein. Given the recognised aetiologic association between oestrogen and breast cancer risk, there is biological plausibility that dietary soy and vegetable intake which is rich in the Chinese diet may have anti-carcinogenic effect on the breast. The seemingly conflicting phenomenon of early age onset and lower BC incidence in China might be due to wide imbalance in the amount of exposure to carcinogenic factor (e.g., HBV infection) for decades and the carcinoprotective exposure levels (e.g., isoflavonoids and flavonoids intake). For example, the increase in carcinoprotective levels would lead to lower incidence of breast cancer and vice versa. Although the focus of this personal view is on HBV, this by no means negates the roles of other known risk factors in breast-cancer development. Characterisation of the role of HBV in BC could potentially benefit Chinese females by decreasing incidence and increasing overall survival.

Keywords: Breast cancer (BC); hepatitis B virus (HBV); flavonoids; Chinese females; estrogen; chronic occult hepatitis
epidemiology of HBV infection in China is still moderate endemic now. Approximately 60% of the Chinese population have laboratory evidence of contact with HBV and 7.2% are chronic carriers of HBsAg (5). In this paper, we propose that HBV infection might have a key role in the early development of BC in China (seen in Figures 1, 2).

**Biology of HBV infection**

In general, the natural history of chronic HBV infection in birth or early childhood is divided into five phases as follows: high replicative low inflammatory, immune clearance, HBeAg (-) chronic, non-replicative, HBsAg loss/occult hepatitis B (6). It is important to note that these stages of infection are not static or always sequential, with possibility of moving from one phase to another in any direction. Viral replication and development of liver disease depend on the balance between viral mechanisms promoting persistence, and host immune control (7). Understanding of the timing of acquisition of the infectious agent in early life and cancer development later in life is unclear. HBV is acquired in early life, during in utero, infancy, early childhood, and adolescence, have a long latency period in human carcinogenesis, but this relationship of HBV with BC is largely unexplored yet.

The vast majority of acutely infected adults are able to spontaneously “clear” the virus from the blood (8). However, in most cases, those individuals who have cleared HBsAg still maintain a low level of infection throughout their lives, especially various cases have been reported of the persistence of HBV as occult infection in BC patients with frequent reactivation during immunosuppressive therapy (9). The, mutations in the pre-S/S genome region of HBV also results in reduced production of HBsAg (10). Apart from mutations within the HBV genome, there are other mechanisms that the virus may utilize to evade an immune response also further by infection of host immune cells, such as peripheral mononuclear cells, integration into the host genome, formation of HBV-containing immune complexes and modulating the host immune response directly (11). In addition to suppressing innate immune responses, the use of a transcriptional template [covalently closed circular DNA (cccDNA)] sequestered in the nucleus of infected cells may allow the virus to evade detection by the innate immune system (12). On the other hand, secreted HBeAg or even HBsAg are also proposed to be

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**Figure 1** The direct and indirect mechanism through which HBV may play a role in causing breast carcinoma. HBV, hepatitis B virus.
viral immunosuppressors to induce the exhaustion of helper T cells (13). Notably, HBV relies on retroviral replication strategy (reverse transcription from RNA to DNA), and eradication of HBV infection is rendered difficult because the stable, long enduring, cccDNA becomes established in hepatocyte nuclei and HBV DNA becomes integrated into the host genome (14,15). Surprisingly, HBV may retreat into immunologically privileged places from where it can seed the circulation and reach cytotoxic T lymphocytic (CTL) inaccessible tissues or even cause immunosupression, thereby maintaining the CTL response in apparently cured individuals and thus prolonging the liver disease to chronic HBV hepatitis (16).

In general, occult blood infection represents the window period of acute infection, persistence of low level replication after recovery, or the occurrence of an escape mutant undetected by current HBsAg assays (17). The occult HBV infection (OBI) prevalence in Asian population, is much higher and ranges from 7.5–16%. In several groups, the prevalence of OBI would be even greater if the tests were performed on liver tissue (18,19). Adult patients with HBV genotype C has been reported to achieve HBeAg seroconversion at an older age than genotype B (20). In China, HBV genotype C is highly prevalent which shows that Chinese females are more prone to late seroconversion and viral persistence when infected with HBV. Studies have shown that occult HBV may be transmitted between relatives or transmitted to children from occult infected or HBsAg-positive mothers (21,22). Additionally, recovery from an acute self-limited hepatitis B may also be followed by OBI, which could last greater than 2–3 decades (23).

Hence, OBI must be more widespread in Chinese populace as HBV being endemic in China and it remains undetectable for decades.

**Possible role of HBV as a carcinogenic factor for BC**

**The indirect role of HBV as a carcinogenic factor for BC**

After family history, lifetime cumulative exposure to reproductive hormones, especially oestrogen, is the most important risk factor for BC. Longer a woman is exposed to cycling reproductive hormones, higher her risk of developing BC (24,25). Although HBV is considered as a non-direct cytopathic virus, persistent and prolonged HBV infection in the liver is accompanied by histological signs of mild hepatic necro inflammation (26), which leads to increase in the level of estrogen as its mainly deactivated in the liver. There have been number of studies on the role of estrogen in causing BC (27-30). Hence, increased exposure of free estrogen due to long term liver dysfunction may lead to breast carcinoma (seen in Figure 1).

HBV is considered to be a sex hormone responsive virus in essence, regardless of the sex disparity in host immune responses (31). ERα (an estrogen receptor) mediates in the transcriptional downregulation of NTCP gene (32) (NTCP is a major receptor for infectious entry of HBV). NTCP gene downregulation was confirmed to be 2-fold lower in female rats than in males (33). This might be one plausible mechanism for estrogen/ERα axis as an anti-HBV guardian to restrict viral infection or spread in liver tissues of Chinese females.
females (34). The seroconversion from HBeAg to anti-HBe and from HBsAg to anti-HBs is also observed more frequently in females than in male subjects (35). Thus, for HBV infection, immune clearance of serum HBeAg and HBsAg is achieved in a higher percentage of female HBV patients than males, which shows that, estrogen paves HBV to remain in occult state and reactivate further with the return of favourable condition.

Estrogens are known to be potent repressors of IL-6 production (36). It can also repress transcription of HBV genes by up-regulating ER-α, which interacts with and alters binding of HNF-4α to the HBV enhancer (37). Thus, estrogen decreases viral load as well as inhibit immune system from attacking the virus. Hence with this delicate balancing action of estrogen by inhibiting virus and immune system leads to increased tendency of developing occult HBV in females.

ERα which is activated by estrogen in the persistently injured stage of liver, also restrains reactive oxygen species (ROS)-mediated cytotoxicity by inhibiting the activation of NF-κB, leading to the suppression of NADPH oxidase activity and decrement of ROS production (38). Due to the defensive effect of estrogen pathway the HBx ability to disturb the redox potential of mitochondrial transmembrane and enhance ROS generation was attenuated in females. These findings suggest that estradiol has a hepatoprotective effect with anti-inflammatory action, by inhibiting proinflammatory cytokines. Hence, long term exposure to estrogen in female HBV carriers was associated with a lower risk of HCC development (39,40). Conversely, it may promote early development of breast carcinoma with long term raise in estrogen levels due to its decreased deactivation thanks to decades of on/off chronic occult necro-inflammatory damage to liver by HBV (seen in Figure 1).

A Taiwan study recently reported that HCV infection, instead of HBV infection, appeared to be associated with the risk of early onset of BC in areas endemic for HCV and HBV (4). Wong et al. (41) reported that screening all patients for HBsAg before adjuvant chemotherapy for BC would prevent a significant number of HBV reactivations, and would likely be moderately cost-effective, which might point to the fact that HBV infection and BC coexist more often and are closely related.

Meanwhile it is well known that a number of in vitro studies have clearly demonstrated that the HCV “core” protein strongly inhibits HBV replication (42) and OBI infection is commonly observed in patients coinfected with non-occult HCV (43). A study reported 50% of chronic HCV patients had previous exposure to HBV in the form of anti-HBc (44). Surprisingly, anti-HBe is well accepted as the most sensitive marker in evaluating HBV infection history. Nearly 100% of chronic HBV subjects and more than 90% of individuals with OBI are HbcAb positive (45,46). Also studies have shown that HCV RNA as a significant predictor for OBI (47), which clearly shows that HCV and HBV infection often coexists together. Furthermore, inhibition of HBV replication and reduction in HBsAg synthesis have been reported in coinfection with HCV (48). The underlining molecular mechanism responsible for this suppressive effect are being extensively studied both in vitro (49) and in vivo studies (50). Therefore, with this fact it must be noted that Taiwan’s clinical study have not included the occult HBV cases which might be present in large numbers of HCV infected patients. Henceforth, the difference in sensitivity and/or specificity of methods used in it could be responsible for the discrepant findings.

Possible role of HBV as a direct carcinogenic factor for BC

HBV-DNA integration into host genome occurs at early steps of clonal tumor expansion and induces both genomic instability and direct insertional mutagenesis of diverse cancer-related genes. HBV DNA is proved to be found in low concentration in the breast milk (51,52). It has also been detected in peripheral blood mononuclear cells (PBMC) and extrahepatic tissues such as, bone marrow cells, spleen, and lymphoblastoid cell lines (53). Also much evidence indicates that HBV may maintain its pro-oncogenic role even in occult infection (54).

The breast oncoprotein hepatitis B X-interacting protein (HBXIP), a conserved 18 KDa protein, is originally identified by its interaction with the hepatitis B virus X protein (HBX) which decreases HBV replication together with HBsAg and HBeAg synthesis, and is believed that the effect is mediated by inhibition of HBX action on the endogenous viral core promoter/enhancer elements (55). High HBXIP expression is predominantly observed in BC tissues instead of the adjacent normal breast tissues and plays a crucial role as a key oncoprotein in the development of BC (55–63). HBXIP also activates the transcriptional coregulatory protein, LIM-only protein 4 (LMO4) through transcription factor Sp1 to promote the proliferation of BC cells (60). It also enhances the angiogenesis and growth of BC cells through modulating fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) (64).

It further enhances the growth and migration of BC cells...
through S100A4 in vivo and in vitro (58) and upregulates Lin28B, thus promoting BC (55) (seen in Figure 1).

**Carcinoprotective factors in Chinese diet and breast carcinoma**

Flavonoids are a family of polyphenolic compounds synthesized by plants with a similar structure, are divided into subclasses, including anthocyanidins, flavanones, flavonols, flavones and isoflavones (65). Soy phytoestrogens, such as genistein, daidzein, and glycitein, are isoflavonoids closely related to human 17β-estradiol (66), but with lower estrogenic activity (67). Several beneficial properties have been attributed to these dietary compounds, including antioxidant, anti-inflammatory, and anti-carcinogenic effects. Phytoestrogens such as genistein, resveratrol, bakuchiol and quercetin have anti-proliferative effects on BC cells (68,69). A favoured mechanism by which soy isoflavones may influence BC development is via their affinity and competition with endogenous oestrogens and other substrates in binding with oestrogen receptors (ERs). Surprisingly it was also reported that S-equol had a greater affinity for ER than its precursor isoflavone daidzein (70), which has a demethylating effect on the CpG islands in the promoters of BRCA1 and BRCA2 genes (71) thus suppressing familial type BRCA gene mutations related breast carcinoma.

It has also been reported that dietary intake of mushrooms and green tea decreased BC risk (72). It is found in vitro studies that tea polyphenols inhibit aromatase, the key enzyme converting androgens to estrone or estradiol (73) and is associated with reduced levels of estrogens, estrone, and estradiol among pre- and postmenopausal Chinese women (73,74). Henceforth it's a proven fact that Chinese diet rich in soy, vegetables, green tea and fruits plays a carcinoprotective role, thus, reducing incidence of BC in Asia (75-77) (seen in Figure 2).

**Stark imbalance in the exposure of carcinogenic and carcinoprotective factors leads to early development of BC**

Breast tissue is highly dynamic throughout a woman's life, and during any of these unique stages, a disruption in the delicate balance of growth factor and hormonal signaling between the stroma and epithelium has the potential to promote disease. The wide imbalance in the amount of exposure to carcinogenic factor (e.g., HBV infection) for decades which belittles the effect of carcinoprotective factors may lead to early development of BC (seen in Figure 2). Controversially, imbalance in the amount of exposure to carcinoprotective factors for decades which belittles the effect of carcinogenic factor (e.g., HBV infection) may lead to low incidence of BC in China.

**Controversies regarding HBV vaccine efficacy and its possible consequences**

HBV vaccine has led to significant reduction in viral transmission. The nationwide vaccination program since 1992 has changed the epidemiology of HBV infection in China from being highest to moderately endemic at present. Erstwhile, It is reported that the efficacy of vaccine is not 100% and is protective for at least 10 years (78). Studies from various countries have detected HBV chronic infections among immunized infants and children (79-83). Many factors may be related to occult infection in vaccinated children, such as hypo-response to HBV vaccination, declining antibody titers, escape mutations in S gene, or high maternal viral loads (84). Additionally, it seems that HBV infection risk is increased dramatically during adolescence. The cumulative 10-year HBV infection risk in postnatal passive active HBV vaccinated subjects was less than 15% (85). On the contrary, more than 30% of these high-risk subjects were HBcAb positive at 15 years of age (86) which indicates the possibility that, with increasing age HBV shows the trend in which it can retain undetected in the dormant state all along the normal efficacy of vaccine and reappear slowly into active state when the vaccine efficacy seems to fade away.

**Conclusions**

The seemingly conflicting phenomenon of early age onset and lower BC incidence in China might be due to wide imbalance in the amount of exposure to carcinogenic factor (e.g., HBV infection) for decades and the carcinoprotective exposure levels (e.g., isoflavonoids and flavonoids intake) (seen in Figures 1,2). In other words, the increase in carcinoprotective levels and decrease in carcinogenic levels would lead to lower incidence of breast cancer and vice versa. Thus understanding the role of chronic occult HBV and its relation with the estrogen could lead to new ways of prevention, diagnosis and adjuvant therapeutic treatments,
and potentially benefit females by decreasing the incidence and increasing overall survival. Also, screening and monitoring of HBV and its vaccine efficacy should be enhanced from neonatal period, which can probably prevent or delay the occurrence of breast carcinoma.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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