Cancer Evo-Dev, a novel hypothesis derived from studies on hepatitis B virus-induced carcinogenesis

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ABSTRACT

Non-resolving inflammation, which may be maintained by infection, pollution, and metabolic stimulants and their interactions with immunogenetic predisposition, provides a fertile field for cancer development. This is strongly evident in hepatocellular carcinoma. Here, the framework of a hypothesis called Cancer Evo-Dev is presented, based on the advances in hepatitis B virus-induced hepatocarcinogenesis. Several aspects central to this theory are as follows: (1) immune imbalance caused by the interaction of immunogenetic predispositions and hepatitis B virus infection maintains non-resolving inflammation; (2) active inflammation executants promote mutations in viral and host genomes via disbalancing mutagenic forces including cytidine deaminases and mutation-repairing forces including uracil-DNA glycosylases, thus promoting cancer-related somatic mutations and viral mutations; (3) a small percentage of the cells whose somatic mutations alter the survival signalling adapt to the inflammatory microenvironment, de-differentiate via demethylating role of cytidine deaminases, and reversely develop into tumor-initiating cells (TICs); (4) under the cultivation of some factors like POSTN from tumor-infiltrating fibroblasts and M2 macrophages, TICs acquire the stemness, cancer-stem cells obtain distinct metastatic and drug-resistant potentials under the selection pressure from distinct microenvironments; (5) glycolysis persistence in the presence of oxygen provides essential energy for cell survival and the raw material for DNA synthesis. Thus, cancer development is characterized by an evolutionary process of “mutation-selection-adaptation”. The framework of Cancer Evo-Dev can be verified in other cancers. Cancer Evo-Dev lays theoretical foundation for understanding the mechanisms by which inflammation promotes cancer development, and it also plays a role in specific prophylaxis, prediction, and targeted treatment of cancers.

NON-RESOLVING INFLAMMATION AND HEPATOCELLULAR CARCINOMA

Inflammation, firstly characterized as “heat, redness, pain, and swelling” by a Roman physician Cornelius Celsus, is a complex biological response to harmful stimuli such as infections and tissue damage. Inflammation can be classified into acute inflammation and chronic inflammation. Acute inflammation, also
termed as resolving inflammation, is an initial stage of inflammation mediated through activation of innate immunity; it lasts for short period. Chronic inflammation, also termed as non-resolving inflammation, is the second stage of inflammation that persists for a long period of time. Chronic inflammation may develop from acute inflammation if the stimuli are not eradicated or inflammation appears with a chronic process, reflecting the weak but active nature of host immunity. Although the two kinds of inflammation are closely linked to form a correlative antagonistic unity, inherent mechanisms regarding proinflammatory molecules, types of infiltrating macrophages, and inflammatory pathways are distinct, as shown in Figure 1[1]. Acute inflammation is often regarded as therapeutic inflammation to ward off infections and/or to repair the tissue damage; whereas chronic inflammation is now considered as pathogenic, being closely linked with most chronic illnesses, such as cancer, cardiovascular diseases, diabetes, obesity, pulmonary diseases, neurologic disorders, and even depression[2]. Non-resolving inflammation is the prerequisite for the development of most cancers. For examples, chronic hepatitis B or C, chronic bronchitis, chronic colitis including ulcerative colitis, chronic cervicitis, chronicatrophic gastritis, and chronic esophagitis (gastroesophageal reflux disease - caused Barrett’s esophagus) often precedes liver cancer, lung cancer, colorectal cancer, cervical cancer, gastric cancer, and esophageal cancer, respectively. Non-resolving inflammation is clearly evident in the development of hepatitis B virus (HBV)-induced hepatocellular carcinoma (HCC). It is generally believed that oral-administered antiviral therapy decreases the risk of developing HCC in patients with chronic hepatitis B (CHB)[3]. However, the risk of developing HCC is significantly higher in the oral nucleos(t)ide analogues-complete responder group compared with the inactive CHB group, regardless of the presence of baseline liver cirrhosis[4], indicating that continuous active inflammation in liver facilitates the development of HCC. Although surgical technologies for the treatment of liver cancer have been improved, postoperative prognosis remains to be precisely evaluated[5-7]. Active inflammation on chronic inflammation background, as reflected by an Ishak hepatic inflammation score (> 6), a higher neutrophil-to-lymphocyte ratio (> 5), and a higher C-reactive protein in sera (> 0.3 mg/dL), etc., also indicate a poor postoperative prognosis such as postoperative recurrence and shorter recurrence-free survival in HBV-related HCC (HBV-HCC) patients[8,9]. Nuclear factor-κB (NF-κB) and signal transducers and activators of transcription 3 (STAT3) are two most important transcription factors involved in inflammatory pathways that play predominant roles in carcinogenesis, especially in HBV-induced hepatocarcinogenesis[10,11]. Thus, inflammatory microenvironment including proinflammatory molecules, tumor-associated fibroblasts, and tumor-associated immune cells with altered expression of the inflammatory pathways facilitates the evolution and development of cancers.

**Maintenance of chronic HBV infection and hepatic inflammation**

Chronic transformation of HBV infection relies on three aspects: infection occasion, the characteristics of HBV genotypes, and genetic predisposition of the key immune molecules. HBV infection in early childhood
is generally believed to be one of the major causes of chronic HBV infection in adulthood. Of the infants born to hepatitis B surface antigen (HBsAg)-positive mothers globally, 42.1% who did not receive HBV passive-active immunoprophylaxis and 2.9% of infants who received the immunoprophylaxis acquired HBV infection perinatally. The perinatal infection occurred in 84.2% and 8.7% of infants born to hepatitis B e-antigen (HBeAg)-positive mothers who did not and did receive immunoprophylaxis, respectively. The infection rates were 6.7% and 0.4% for infants born to HBeAg-negative-carrier mothers, respectively. Moreover, the chronicity rates of HBV infection acquired perinatally were 28.2% in infants born to HBeAg-negative mothers and 64.5% in infants born to HBeAg-positive mothers\(^{[12]}\). This is possible due to the fact that the immaturity of immune system in infants make it unable to recognize HBV as an external antigen, thus establishing chronic HBV infection although the immune response can be aroused thereafter. Clearly, perinatal HBV infection is an important but not the predominant cause of chronic HBV infection in adulthood. Chronic transformation of acute hepatitis B caused by horizontal transmission among adolescents and adults contributes to the remaining proportion of chronic HBV infection. Approximately 8.5% of acute hepatitis B in adults in Shanghai, China, develops into chronic HBV infection 6 months after acute infection\(^{[13]}\). In mainland China where genotypes B (B2) and C (C2 and C1) are endemic, HBV subgenotype B2 is more apt to causing acute infection because of higher viral load in the virus-providing chronic carriers whereas HBV subgenotype C2 is more apt to causing chronic transformation following an acute course\(^{[13,14]}\). HBV C2 is more likely to develop liver cirrhosis and HCC than does HBV B2, the two major HBV subgenotypes endemic in China, possibly because of the stickiness nature of HBV subgenotype C2\(^{[13-17]}\). The third most important cause of chronic HBV infection and active inflammation is the genetic predispositions of some key immune and proinflammatory molecules. Genome-wide association study in the eastern Asian populations have shown that a total of 11 single nucleotide polymorphisms (SNPs) in genetic loci including HLA-DPA1 and HLA-DPB1, some SNPs in genetic loci including HLA-DQ and -DR, and a locus near HLA-Care significantly associated with CHB\(^{[18-22]}\). Interestingly, these SNPs in the loci encoding human leukocyte antigen-class II (HLA-II) are also significantly associated with vaccine response as well as the risks of acute-on-chronic liver failure, HBV-related liver cirrhosis, and HBV-associated HCC\(^{[23-27]}\). Interestingly, different human races have different allelic frequencies of SNPs that affect the expression of HLA-DP, HLA-DQ, and the inhibitory component of NF-κB complex IkBa gene NFKBIA. These genetic loci whose dominant alleles are significantly association with increased risks of chronic progression of HBV infection (or whose rare alleles are significantly associated with decreased risks of chronic HBV infection) include rs3138053 (affecting NFKBIA), rs2856718, rs7453920, and rs9275319 (affecting HLA-DQ), and rs9277378, rs2395309, rs2301220, and rs9277341 (affecting HLA-DP)\(^{[12]}\). The polymorphic genotypes that are significantly association with increased risks of chronic progression of HBV infection as well as immune selection of the end-stage liver diseases-associated HBV mutations are more frequent in the Han Chinese than in European populations. These data indicate that the Han Chinese are inherently more apt to progressing into chronic infection once exposed to HBV infection than European, whereas European tend to recover from HBV infection spontaneously\(^{[12]}\). This might be one of the reasons why chronic HBV infection and the HBV-induced end-stage liver diseases are more frequent in Chinese than in European populations. The HLA-II genetic polymorphisms may predispose immune imbalance upon HBV infection, impair immune function for HBV clearance, and maintain chronic HBV infection and hepatic inflammation, and thus facilitating the progression of CHB into liver cirrhosis and HCC.

**“Dead-end” evolution of HBV**

During HBV-induced hepatocarcinogenesis, both of hepatic cells and the viruses experience the process of evolution. Viral evolution serves as a valuable clue to investigate the mechanism underlying HBV-induced hepatocarcinogenesis. HBV belongs to the Hepadnaviridae family found in both mammals (orthohepadnaviruses) and birds (avihepadnaviruses) and is highly conserved in their host species during the long-term evolutionary process. Although primate hepadnaviruses are indigenous to their hosts, hepadnaviruses isolated from apes are grouped as HBV genotypes in phylogenetic analyses. With only 5% divergence from the chimpanzee viral isolates, the isolates from gorilla are categorized in the HBV genotypes. Avihepadnaviruses are the most distant relatives of HBV with a nucleic acid homology of only 40%. Compared to avihepadnaviruses, Woodchuck hepatitis virus and ground squirrel hepatitis virus as mammalian hepadnaviruses are more closely related to HBV and differ by only 17%. Genetic evolution analysis indicates that HBV and orthohepadnaviruses from non-human primates are phylogenetically clustered in the same evolutionary branch\(^{[28]}\). These evidences indicate that members of Hepadnaviridae family are highly conserved in their evolutionary history. However, HBV experiences a relative rapid evolution in their genome since chronic HBV infection is established in a subset of infected populations.

Previous researches by our group identified the wild-type HBV sequences (so-called the standard...
sequences) of HBV subgenotypes B2 and C2, based on the whole HBV genome sequenced using approximately 1,000 asymptomatic HBsAg carriers from community-based epidemiological surveys in the Yangtze river delta region of mainland China. Based on the wild-type HBV sequences, we subsequently characterized the end-stage liver diseases-related mutations and their development patterns in HBV subgenotypes B2 and C2. We found that the HBV mutations posing a significant risk of HCC or liver cirrhosis were mainly located within the enhancer II/basal core promoter/precore (EnhII/BCP/PreC) and preS regions of HBV genome\(^\text{[29-31]}\). We summarized the data concerning the association of the HBV mutations and HCC risk published up to 2009, and found that the frequencies and locations of the HBV mutations accumulate consecutively during the "trilogy" of HBV-induced carcinogenesis (CHB, liver cirrhosis, and HCC) and that these HBV mutations can be applied to predict the occurrence of liver cirrhosis and HCC\(^\text{[32]}\). In our prospective cohort study, we have identified the baseline HBV mutations (C1653T, A1762T/G1764A, and T1753V) increase the risk of HCC both independently and "dose-dependently". The so-called "dose-dependently" is referred to that the HCC risk is significantly higher in the CHB patients carrying one of the three mutations than in those without the mutation, is significantly higher in those with two of the three mutation than in those with one of the three mutation, and is also significantly higher in those with all the three mutation than in those with two of the three mutations. Thus, the baseline HBV mutations in combination are able to predict the occurrence of HCC in CHB patients\(^\text{[33]}\). Several longitudinal studies carried in China have also demonstrated that baseline A1762T/G1764A mutation increases the risk of HCC in chronic HBV carriers or CHB patients\(^\text{[34-37]}\). Among the HCC-risk HBV mutations, the A1762T/G1764A is usually detected in the early stage in young adolescents, while other mutations including T1753V, C1653T, G1899A, and preS deletion appear only at the late stage of chronic HBV infection\(^\text{[12,38]}\). Reaction to chronic HBV infection, as characterized by immune response-induced hepatocyte damage and release of transaminase, facilitates the generation of the HBV mutations, indicating active immune selection of the HBV mutants during HBeAg seroconversion from HBeAg-positive to HBeAg-negative. One of the main features of HBV mutations is a deficiency of the CD8\(^+\) T-cell epitope, a consequence of immune selection. Reduction of CD8\(^+\) T cell epitopes of HBV is one of the common strategies to evade immune eradication. HBV that has a low density of CD8\(^+\) T cell epitope in their core and X proteins are selected during long-term evolution\(^\text{[39]}\), thus CD8\(^+\) T cells play an important role in the immune selection of HCC-related HBV mutants [Figure 2].
natural mutations are therefore constrained to special regions of the HBV genome.

The inflammatory microenvironment of liver tissue is therefore necessary for the co-evolution of HBV and the host genome[48]. Although tumor-adjacent tissues are pathologically categorized as inflammatory hepatic tissues, they are typical precancerous lesions and have already reached the middle stage of HCC evolutionary process because somatic mutations might occur in the inflammatory liver tissues. Somatic mutations in given genomic locations will become the driving mutations if they confer growth advantage of the mutated hepatocytes. The HCC that relapses more than 2 years after curative resection is believed to be mostly recurrent HCC and not from a spread of the initial HCC cell diffusion into the remnant liver tissue. The species and frequencies of certain HBV mutations in adjacent tissues are different in HBV-infected patients with HCC (HBV-HCC patients) with distinct postoperative prognosis. Together with immune markers and expression levels of inflammatory genes in adjacent hepatic tissues, the HBV mutations can be applied to predict postoperative prognosis in HCC patients[46]. The HBV mutations in the EnhII/BCP/PreC region such as A1762T/G1764A in the remnant liver after curative surgery or in the circulation before liver transplantation have been proven to be predictive markers for postoperative survival and HCC recurrence, although this result has not been repeated by some research groups[47-49]. This indicates that HBV evolution in adjacent tissues continues until the patient dies. Antiviral therapy that can attenuate viral replication and subsequent hepatic inflammation notably promotes postoperative prognosis of HBV-HCC patients[50-53], possibly because antiviral treatment can block HBV evolution in adjacent hepatic tissues and also likely in remaining HCC tissues.

Thus, Hepadnaviridae family members, including HBV, are highly conservative across species with distinct but connected evolutionary background. Wild-type HBV has the advantage of infecting hepatocytes of new hosts, facilitating viral spread from one individual to another, and contributing to the maintenance of viral species. The HCC-related HBV mutants can cause or promote malignant transformation, but might have lost the advantage of person-to-person transmission. Those mutants are therefore eliminated if their host individuals died of the end-stage liver diseases including HCC, which is termed “dead-end” evolution of HBV.

The HCC-related HBV mutated X or large S fragments promote the malignant phenotypes
As described above, the baseline HBV mutations including A1762T/G1764A, C1653T, and T1753Vin the 3' terminal of HBx gene in sera “dose-dependently”
predict the occurrence of HCC in longitudinal studies, especially cohort studies\textsuperscript{[33-37]}. The frequency of HBV preS deletion also increases consecutively from ASCs to HCC and preS mutation is associated with a 3.77-fold increased risk of HCC\textsuperscript{[32]}. The predictive value of HBV preS deletion on the occurrence of HCC in patients chronically infected with HBV has been confirmed in a prospective study\textsuperscript{[54]}. Recent deep sequencing analysis has demonstrated that the preS deletions involving a specific fragment (nt2977-3013) in HBV genotype C are significantly associated with HCC\textsuperscript{[55]}. These epidemiological evidences indicate that the HBV mutations including preS deletion, A1762T/G1764A, C1653T, and T1753V are the etiological factors of HBV-induced HCC. Experimentally, in vitro transfection with the HBx mutants with changes that correspond to A1762T/G1764A, T1753A, T1768A, or a combination of these (combo) showed that the combo mutant decreased levels of p21, increased cyclin E expression, and increased expression of S-phase kinase-associated protein 2 (SKP2) in primary human hepatocytes and HepG2 cells. The combo mutant accelerated p21 degradation and cell cycle progression in HepG2 cells. Thus, HBx mutants with changes that correspond to a combination of the core promoter mutations up-regulate SKP2, which then down-regulates p21 via ubiquitin-mediated proteasomal degradation. The core promoter mutations might increase the risk of HCC by this pathway\textsuperscript{[56]}. Transfection of full-length HBV genome with the core promoter mutations in combination also up-regulated SKP2 expression via activating the E2F1 transcription factor and in turn downregulated cell cycle inhibitors, thereby accelerating cellular proliferation\textsuperscript{[57]}. Mutations in the preS and S regions also notably facilitate carcinogenesis. Transfection of HuH7 cells with the large S region with preS deletion has shown that HCC-associated single-nucleotide variants (SNVs) in the small surface region of HBV genome influence carcinogenesis pathways, including endoplasmic reticulum-stress and DNA repair systems\textsuperscript{[58]}. The HBV large envelope protein gene fragment (preS1/preS2/S), with F141L mutation in the preS2 region, can significantly promote the proliferation of hepatocytes by downregulating the p53 and p21 pathways and upregulating the expression of cyclin-dependent kinase 4 and cyclin A. The colony-forming rates of hepatocytes expressing F141L-large envelope protein are about twice as high as those expressing the wild-type HBV large envelope protein\textsuperscript{[59]}. Random integration of HBV DNA into the host genome is present in HBV-infected subjects. If the integration events endow the hepatocytes with growth advantage, the integration might facilitate the development of HCC, therefore, have the opportunity of being recorded. HBV integration is common in HBV-HCC, leading to the truncation of the HBV genome, particularly at the C terminus of HBx (Ct-HBx)\textsuperscript{[59]}. Ct-HBx can enhance cell invasiveness and metastasis of HCC in a manner that is more potent than that evoked by full-length HBx and often predict the poor postoperative prognosis and ineffectiveness of antiviral prophylaxis for HCC recurrence\textsuperscript{[61,60]}. These evidence indicates that some HBV X mutants and large S mutants can promote the development and progression of HCC.

**Interaction of genetic predispositions of immune or inflammatory molecules with the HBV mutations in HBV-induced hepatocarcinogenesis**

As described above, the HLA-II genetic polymorphisms are statistically associated with the outcomes of exposure to HBV\textsuperscript{[18-27]}. This genetic background may predispose immune imbalance upon HBV infection. In our previous epidemiological studies, we found that the HLA-II genetic polymorphisms are statistically associated with the generation of the liver diseases-associated HBV mutations. The HLA-DP polymorphisms rs3077 (CT + TT vs. CC), rs3135021 (GA + AA vs. GG), rs9277535 (GA + AA vs. GG), and rs2281388 (CC vs. CT + TT) significantly decrease HBV persistence in genotype B HBV-infected subjects; HLA-DP genotypes that promote HBV clearance are associated with a lower prevalence of HBV mutations increasing HCC risk (C1653T, T1674C/G, A1846T, G1896A, preS2 mutations, and preS deletion in genotype C) and a higher prevalence of HBV mutations decreasing HCC risk (G1652A, T1673C, T1674C, G1719T, G1730C, and G1799C in genotype B and A1727T in genotype C); furthermore, significant effects of HBV mutations on cirrhosis and HCC are selectively evident in those with the HLA-DP genotypes that promote HBV persistence\textsuperscript{[61]}. Thus, the HLA-DP polymorphisms affect genotype B HBV clearance, regulate immune selection of viral mutations, and influence cirrhosis and HCC risks contributed by the HBV mutations. In addition, HLA-DQ genetic polymorphisms rs2856718 variant genotypes are significantly associated with an increased frequency of HBV A1726C mutation, a cirrhosis-risk, HCC-protective mutation, in genotype C; a rs9275319 variant genotype (GG) is significantly associated with an increased frequency of preS1 start codon mutation, an HCC-risk mutation, in genotype C. Thus, the HLA-DQ polymorphisms affect the risks of cirrhosis and HCC differently in chronic HBV-infected subjects, possibly via interacting with the HBV mutations\textsuperscript{[63]}. As NF-κB and STAT3 are two most important inflammatory pathways\textsuperscript{[15,11]}, their genetic predispositions affecting the expression of both signaling pathways may play roles in HBV-induced hepatocarcinogenesis. We have demonstrated that STAT3 SNP rs2293152 (GG vs. CC) is significantly associated with HCC risk compared with the
subjects without HCC. Compared with HCC-free HBV-infected subjects, rs2293152 GG is solely associated with HCC in women. This genotype is significantly associated with high viral load (≥ 1 × 10⁴ copies/mL) and increased frequencies of T1674C/G and A1762T/G1764A. Multiplicative interaction of STAT3 rs1053004 with T1674C/G significantly increases HCC risk, whereas rs2293152 and A1726C interaction reduces it, adjusting for covariates including HBV mutations in the EnhII/BCP/PreC region; the interaction of rs4796793 with preS2 start codon mutation significantly increases HCC risk, adjusting for covariates including HBV mutations in the preS region. Thus, STAT3 SNPs appear to predispose the host with HBV mutations to hepatocarcinogenesis[63]. We have also demonstrated that genetic polymorphisms improving NF-κB activity contribute to genotype B HBV clearance. In the genotype C HBV-infected subjects, variant genotypes of rs2233406 (NFKBIA-826C>T) are significantly associated with an increased risk of HCC compared with HCC-free HBV-infected subjects and significantly increase the frequencies of HCC-related HBV mutations including A1762T/G1764A, T1753V, preS1 start codon mutation, and preS deletion; Del allele of rs28362491 (NFKB1-94Ins>Del) significantly increase the frequency of A1762T/G1764A and reduce the frequency of preS2 start codon mutation. The variant genotypes impair NFKBIA promoter activity in hepatic cells. The interaction of rs2233406 variant genotypes (CT + TT vs. CC) with A1762T/G1764A significantly increase the risk of HCC in genotype C HBV-infected subjects[64,65]. These lines of evidence imply that immunogenetic polymorphisms may predispose chronic transformation of HBV infection, increase the frequencies of viral mutations via activating cytosine deaminases, and facilitate immune selection of HCC-causing HBV mutations via arousing active but not effective immune response against the pathogen.

DEVELOPMENT AND EVOLUTION (DEV-EVO), A NOVEL HYPOTHESIS RELATED TO CARCINOGENESIS

Development is referred to the process that a fertilized egg develops into an individual. In humans, the fertilized diploid cell composing of paternal haploid and maternal haploid differentiates into various functional and/or structural cells to form different organs and tissues of an infant in mother’s uterus within 40 weeks. The developmental process is a succession of functional and morphologic changes from a single cell form (fertilized egg) to a multicellular form (blastocyst), from an aquatic state (living in amniotic fluid) to a terrestrial state (pulmonary respiration). During this process, the founding diploid cell, a progenitor, divides rapidly and gives many other different types of cells via altering their gene expression profiling. The changes in gene expression profiling are achieved by epigenetic modifications such as methylation in the upstream regulatory regions of given genes, rather than altering the primary sequences of these genes. After born, lung takes over the responsibility of gas exchange, some genes only expressed in the embryonic stage are silenced and other genes solely expressed in adult cells are activated, most possibly by some epigenetic modifications. Surprisingly, the developmental process of humans resembles the process of long-term organic evolution morphologically, e.g. from single cell creatures to multicellular creatures, and from aquatic creatures and amphibian to terrestrial mammals [Figure 3]. Furthermore, some evolutionarily conserved molecular networks such as HOX, Hedgehog, and Myc play important roles in the developmental process[66-72], indicating development and evolution share some inherent mechanisms. During the past 20 years, the discovery of conserved gene networks that control embryonic development and the ability to examine genomic records has revolutionized Darwinian evolutionism that animal relationships had to be deduced by observation of external morphological characteristics. The integration between developmental biology and evolution has been named Evo-Devo[73-77]. Dr. Raff pointed out that the evolution of complex organisms such as animals and plants had involved marked changes in morphology and new features had appeared; but evolutionary change occurred not by the direct transformation of adult ancestors into adult descendants but rather when developmental processes produced the features of each generation in an evolving lineage. Thus, evolution cannot be understood if do not understand the evolution of development, and how the process of development itself biases or constrains evolution[75]. Based on these previous work, in combination with previous observations, I like to define Evo-Dev as follows: Evo-Dev is a discipline to investigate the inherent mechanisms by which the short-term developmental process resembles the long-term evolutionary process and to characterize the role of developmental process on the evolution of complex organisms.

Carcinogenesis represents an evolutionary process. It was firstly proposed by Dr. Nowell in 1976 that most neoplasms arise from a single cell of origin, and tumor progression results from acquired genetic variability within the original clone allowing sequential selection of more aggressive sublines; tumor cell populations are apparently more genetically unstable than normal cells, perhaps from activation of specific gene loci in the neoplasm[78]. Cancer clone genetic diversification and sub-clonal selection occurs within tissue ecosystems.
The implicit parallel was to Darwinian natural selection with cancer equivalent to an asexually reproducing, unicellular, quasi-species\textsuperscript{[79]}. In the past decades, especially after widespread application of new generation sequencing, cancer evolution including a reiterative process of clonal expansion, genetic diversification, and clonal selection within the tissue ecosystems have been extensively investigated. Drs. Greaves and Maley\textsuperscript{[80]} outlined key contents of cancer evolution as follows: (1) cancers are characterised by divergent cells of origin and mutational spectra; (2) cancers evolve over variable time frames (about 1-50 years) and tempos and the clonal structure, genotype and phenotype shifts over time; (3) the number of mutations found in any cancer can vary from 10-20 to hundreds of thousands, the great majority are “passengers” and a few are “drivers”; (4) cancers acquire, via mutational (and epigenetic) changes, a variety of critical phenotype traits that compound to empower territorial expansion, via proliferative self-renewal, migration, and invasion; (5) advanced, disseminated or very malignant cancers appear to be almost uniquely competent to evade therapy; and (6) this complexity can be explained by classical evolutionary principles. Most mutational processes have biases at the DNA sequence level and mutational spectra in cancers can reflect or implicate particular error-prone repair processes or particular genotoxic exposures, e.g. cigarette carcinogens, UV light, and chemotherapeutics\textsuperscript{[81]}. We believe that somatic mutations increase with increasing age for two major reasons: (1) accumulation of exposures to various mutagens increases with increasing age; and (2) “mistake” mutations can be spontaneously generated or introduced in each cell cycle. However, the most of somatic mutations do not alter gene expression-defined key functions related to cell growth advantage or resistance to environmental insults. These mutations can be “passenger mutations”. If a somatic mutation endows the cell a growth advantage in a hostile inflammatory microenvironment, it is termed as “driver mutation”. Cells carrying “driver mutation” are positively selected in inflammatory microenvironment, facilitate the cross-talks with proinflammatory cells including tumor-associated M2 macrophages and neutrophils, promotea process termed as epithelial-mesenchymal transition (EMT) and stepwise de-differentiation into tumor-initiating cells (TICs), and adapt to new inflammatory microenvironment [Figure 3]. In our previous study, we found that expression of periostin (POSTN) from tumor-infiltrating fibroblasts significantly promoted the proliferation, anchorage independent growth, invasion, and chemo-resistance of cancer cells; whereas these effects were counteracted via targeting to PI3K/Akt or Wnt/β-catenin signaling pathway\textsuperscript{[82]}. Thus, cancer development represents “mutation-selection-adaptation” evolutionary process in proinflammatory microenvironment, which is quite in accordance with

\textbf{Figure 3:} Synthesis of Dev-Evo and its potential link to carcinogenesis. Dev-Evo: synthesis of development and evolution; EMT: epithelial-mesenchymal transition.
Darwinian evolutionism.

During human embryo development, most genes expressing at this stage will be silenced after birth, but some genes including that encoding α-fetoprotein (AFP), will re-express for a short period of time when liver or testis is injured. Importantly, cancer is characterized as a reverse-developmental process, that is, develop from differentiated cells into undifferentiated cells. Oncofetal proteins are mostly referred to those that normally express at embryonic stage are silenced after birth, and then re-expressed persistently in the circulations of cancer patients. AFP serves as a diagnostic biomarker of HCC. The human homologue of the Drosophila spalt homeotic gene, SALL4, encoding an oncofetal protein Sall4, is one of the key factors for self-renewal and maintenance of embryonic stem-cell pluripotency. SALL4 is expressed in the human fetal liver and silenced in the adult liver, but can be detected in a subgroup of HCC. The re-expression of SALL4 is related to the “stem function” of HCC cells and indicates invasion and unfavourable prognosis\(^{[83-86]}\). As a matter of fact, some cells with stem-cell-like characteristics become the main malignant subgroup in tumor tissues; embryonic or stem-like gene expression signatures expressed in cancers of distinct histotypes including HCC are robustly associated with cancer cell self-renewal, EMT, increased aggressiveness, and poor postoperative prognosis\(^{[87-92]}\). Furthermore, the cell aging process is accompanied by the shortening of telomeres, which does not seem to occur in cancer cells. Telomerase activation occurs through telomerase reverse transcriptase (hTERT) induction. hTERT and ZEB1 form a complex, which directly binds to the E-cadherin promoter, and then inhibits E-cadherin expression and promotes EMT\(^{[93]}\). Sirtuin 1 has been implicated in telomere maintenance and HCC growth. Sirtuin 2, another member of the sirtuin family, plays a role in maintaining the motility, invasiveness, and EMT phenotypes of HCC\(^{[94]}\). In terms of morphology, EMT is the process in which epithelial cells lose their epithelial characteristics and acquire mesenchymal characteristics, structure, and biologic function. An EMT usually occurs at a critical stage of embryonic development, and it is equally important for cancer metastasis\(^{[95]}\). In the process of cancer invasion through EMT, epithelial cells acquire “stemness”, including self-renewal and antiapoptotic capacities. Most tumor cells are differentiated, with limited amplification ability. However, a small proportion of tumor cells with the “stemness” feature becomes the main malignant cell subsets in tumors and is known as cancer stem cells, responsible for the disease’s malignant nature and chemo-resistance. Thus, retro-differentiation or reverse-development is the hallmark in cancer development.

THEORETICAL FRAMEWORK OF CANCER EVO-DEV

Cancers are caused by the accumulation of somatic mutations - a process that abides by the Darwinian evolutionism: mutation-selection-adaptation. Somatic mutation patterns related to chronic inflammation have been identified in most cancers\(^{[96]}\). In some cancers, the inflammation-related somatic mutations increase with time, accompanied by a decline in the mutations related to the initial exposure\(^{[97]}\). Those distribution characteristics and the switch in mutation domination can be analyzed from an evolutionary perspective, suggesting that inflammation sometimes induced by chronic infection might not only cause somatic mutations, but also play an important role in selecting TICs as a cancer-supportive niche. We ever proposed the scientific hypothesis of Cancer Evolution-Development (“Cancer Evo-Dev”) and summarize the basic concepts and theoretical framework\(^{[88-100]}\). Here, some further evidence are presented to optimize the theoretical framework of Cancer Evo-Dev. This novel scientific hypothesis may help in elucidating the mechanisms by which cancer develops and optimizing the most cost-effective ways to control these life-threatening diseases.

Figure 4 depicts the framework of Cancer Evo-Dev exemplified by HBV-induced hepatocarcinogenesis. Evolutionary process of HCC is a succession of important molecular events-from inflammatory precancerous lesions to carcinogenesis, postoperative recurrence, and metastasis. Those events include, but are not limited to viral mutation, epigenetic modification, somatic mutations, and alteration of signaling pathway networks. The synergetic effects of genetic and environmental factors contribute to imbalance of the host immune system, resulting in the activation and maintenance of non-resolving inflammation, thus providing a microenvironment for the process of cancer evolution and development. Under conditions of non-resolving inflammation, activated NF-κB complex and proinflammatory molecules can trans-activate the expression of nucleic acid editing enzymes including APOBEC family of cytidine deaminases, rather than uracil DNA glycosylases (UNG), thus promoting viral and somatic mutations. Actually, the imbalance between mutation-promoting forces like AID/APOBECs and mutation-repairing forces like UNGs is responsible for the generation of somatic and viral mutations\(^{[99]}\). Viral mutants facilitate the malignant transformation of normal hepatic cells via inducing EMT. Most mutant cells are eliminated by selective pressures imposed by the weak but active immune response. Although a small proportion of mutant cells survive in the hostile
Figure 4: Theoretical framework of Cancer Evo-Dev, as exampled by HBV-induced hepatocarcinogenesis. A: The classic diagram of Cancer Evo-Dev. The imbalance between mutation-promoting forces like AID/APOBECs and mutation-repairing forces like UNGs is responsible for the generation of somatic and viral mutations, thus promoting Warburg effect. In addition, AID/APOBECs also demethylate the promoters regions of some transcriptional factors including EMT; B: exposures to some mutagens and HBV infection lead to mitochondria DNA mutations, thus promoting Warburg effect. In addition, AID/APOBECs also demethylate the promoters regions of some transcriptional factors including EMT; C: the diagram of Cancer Evo-Dev explaining why HCC is a male sex-predominant cancer. mtDNA: mitochondria DNA; AID/APOBECs: activation-induced cytidine deaminases/the human apolipoprotein B mRNA-editing enzyme catalytic polypeptide; UNG: uracil DNA glycosylase; EMT: epithelial-mesenchymal transition; HBV: hepatitis B virus; AR: androgen receptor; ROS: reactive oxygen species; Mt: mitochondria; HCC: hepatocellular carcinoma.
inflammatory microenvironment in precancerous lesions. Those surviving mutant clones later evolve into TICs by altering the original cell signal patterns and promoting EMT through epigenetic regulation possibly by APOBECs. Some established cancer markers such as AFP and SALL4 are usually expressed at the embryonic stage, silenced after birth, and re-expressed in cancer patients. The process of cancer development can be characterized as “backward evolution” and “retro-differentiation”.

**KEY ISSUES REGARDING CANCER EVO-DEV**

**Indispensable role of non-resolving inflammation**
It is widely accepted that most solid tumours and some hematologic malignancies are associated with non-resolving inflammation. According to the Darwinian evolutionism and the origin of species, the process of cancer evolution is based on two conditions: the continuous acquisition of somatic mutations and natural selection acting on the resultant phenotypic diversity.

A chronic inflammatory microenvironment serves as a niche for that process by inducing endogenous mutagenic factors such as APOBECs and provides selection pressure. During carcinogenesis, cancer cells must overcome four barriers: (1) the cell-cycle checkpoint that regulates cell division; (2) apoptosis, which limits cell proliferation; (3) telomere length, which determines the total number of cell divisions; and (4) the cell adhesion barrier that prevents cell migration. The non-resolving inflammation can alter the “ecologic” conditions in local and/or systematic tissues, weaken the functions of the above barriers, cause genomic instability via inducing the overexpression of AID/APOBECs, and provide opportunities for backward evolution into cancer stem cells in mesenchymal tissues. In inflammatory microenvironment, inflammatory mediators such as prostaglandin E2, leukotrienes, cytokines, and chemokines are highly induced via autocrine or paracrine modes of action, resulting in abnormal transformation of the tissue microenvironment, infiltration of dysfunctional immune cells, and decreased epithelial integrity, thus promoting cancer evolution. Non-resolving inflammation not only promote the occurrence of cancers of the most histotypes, but also facilitate distant metastasis and the recurrence after the treatment, indicating that non-resolving inflammation promotes the development of cancers in the entire course of cancer evolution.

**APOBECs bridge inflammation and cancer**
The APOBECs, a family of cytidine deaminases, are powerful endogenous mutagenic factors that play critical roles in many biologic processes, especially in innate immunity and humoral immunity. This group of enzymes can specifically catalyze irreversible cytidine and deoxycytidine deamination to convert bases from cytosine to uracil, creating a cytosine-to-uracil mismatch in minus-strand and reverse-transcript guanosine-to-adenosine (G-to-A) transitions in plus-stranded DNA. Activation-induced cytidine deaminase (AID) and APOBEC3 cytidine deaminases were found in the pathways of both the acquired and innate immune responses.

APOBEC3 cytidine deaminases can also hyper-edit HBV DNA and inhibit HBV replication. APOBEC3 proteins are present at low levels in normal liver, but its gene expression is highly stimulated by both IFN-α and IFN-γ. APOBEC3 cleave amino groups from cytidine bases converting them to uracil in newly synthesized DNA following reverse transcription of pregenomic RNA. This modified HBV DNA is susceptible to degradation, or alternatively, numerous G-to-A nucleotide mutations are incorporated into positive-strand viral DNA. This process is counteracted by UNG.

Accordingly, APOBECs family members can also increase somatic mutations to a threshold that exceeds the host’s repair ability, thus initiating the cancer evolutionary process. In AID transgenic mouse models, mutations induced in the TP53 and β-catenin genes by constitutive expression of AID can generate HCC (13.75%), lung cancer (8.75%), and gastric cancer (1.25%). In humans, genetic susceptibility, viral infection, and their interaction contribute to an unbalanced immune system, resulting in chronic inflammation. In the inflammatory microenvironment, the proinflammatory cytokine/chemokine and NF-κB complex are persistently activated, which can significantly increase the expression of APOBECs at the transcription level. The high levels of APOBEC expression can overcome the strength of UNG, APOBECs get the advantage to edit the single-stranded DNAs that are temporarily generated during the transcription and replication process, consequently promoting somatic mutations. If the overall metabolic level exceeds the reserve capacity of the downstream repair pathways, somatic mutations will be further increased. An APOBECs-directed mutagenesis pattern is widespread in human cancers. Significant presence of the APOBEC mutation pattern are evident in bladder, cervical, breast, head and neck, and lung cancers, reaching 68% of all mutations in enrolled tumor samples. The APOBEC mutation pattern also extends to cancer-associated genes, implying that APOBECs-induced mutagenesis is carcinogenic. The spontaneous rate of somatic mutations is not high enough to trigger the evolution process. There must be some mutagenesis-driving forces including defective DNA repair capacity, exogenous or endogenous mutagen exposures, and intrinsic mistakes of DNA replication, which increases the mutation rates in cancer genomes. A distinct mutagenic process generates various mutation combinations.
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termed as “signature”. The APOBECs-related mutation signature is widely prevalent in more than half of all cancer types under investigations, suggesting that the inflammatory response is the common mechanism by which mutations are generated. Even though, the frequencies of somatic mutations in a single gene are not high in the patient population. For example, the rates of mutation in the cording regions of ARID1A and ARID2, two genes with classic HCC-related genetic variations, are 16.8% and 5.6% respectively115. Such a low detection rate of each mutation makes it unable to be applied for the prediction, prevention, early diagnosis, and treatment of cancers. However, somatic mutations in different genes with a similar function can alter a specific signal pathway that is related to the stem characteristic of cancers and therefore promote carcinogenesis. For the most malignancies, so-called driver somatic mutations, mostly at low frequencies in tumor tissues, alter a limited number of cellular signaling pathways through which a growth advantage can be incurred. All of the known driver genes can be classified into one or more of 12 pathways of three major functions: cell survival, cell fate, and genome maintenance. Cell survival contains “cell cycle/apoptosis”, “RAS”, “phosphoinositide 3-kinase (PI3K)”, “STAT”, “mitogen-activated protein kinase (MAPK)”, and “transforming growth factor β (TGF-β)”. “NOTCH”, “Hedgehog”, “APC”, “chromatin modification”, and “transcriptional regulation” contribute to cell fate function. Genome maintenance is governed by “DNA damage control”.112 The combined mutations-affected critical molecules in the signalling pathway networks can be developed as novel diagnostic biomarkers and therapeutic targets. A series of somatic mutations in HBV-induced HCC mainly affect the chromatin remodelling pathways (ARID1A, ARID1B, and ARID2), the p53/RB tumor suppression pathway (IRF2, TP53, and CDKN2A), the Wnt/β-catenin signal pathway (RPS6KA3-AKT1, AXIN1, NFE2L2-CTNNB1), and the Ras/PI3K pathway (PTEN, PIK3CA, KRAS, NRAS).111,113,115 Key genes affecting epigenetic activities including ARID2, encoding a subunit of the polybromo- and BRG1-associated factor (PBAF) chromatin remodeling complex and ARID1A, encoding a component of the SWI/SNF chromatin remodeling complex are most frequent ones. In addition, cell invasion-related factors-coding genes VCAM1 and CDK14, and gene encoding androgen receptor (AR)113,116. Both C>G>A:T and T>A>A:T transversions are frequent among the non-silent mutations114, indicating AID/APOBECs-induced somatic mutation is one of the major mutation patterns. These mutations facilitate the development of HCC via activating some evolutionarily conserved signal pathways, such as PI3K/Akt/mammalian target of rapamycin, NF-kB/TNF-α, Raf/MAPK/ERK, TGF-β1, Jak, Wnt/β-catenin, and STAT3/interleukin 6 (IL-6)117,118 and also indicate that Wnt/β-catenin signaling may cooperate with both oxidative stress metabolism and Ras/MAPK pathways in hepatocarcinogenesis113. ActivatedSTAT3/IL-6 and NF-kB/TNF-α can induce hepatocytes to lose their epithelial characteristics (EMT) and initiate backward evolution. TGF-β1 can facilitate EMT, which can be enhanced by IL-6 and TNF-α. The synergistic effect of those three cytokines can promote the transformation of normal hepatocytes into stem-like cells. Antiviral therapy can significantly reduce the risks of occurrence and postoperative recurrence by HCC via relieving hepatic inflammation50-53,119, possibly because termination of inflammation can destroy the fertile environment for cancer evolution.

**AID/APOBECs-regulated demethylation and EMT are important in malignant transformation**

AID/APOBECs not only promote somatic hypermutation but also regulate gene expression epigenetically by directly deaminating 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) in concert with base-excision repair to exchange cytosine, thus promoting gene demethylation and removing epigenetic memory to stabilize the pluripotent state in embryonic stem cells120,121. EMT, a driving force behind the development of cancers, in its various forms is driven by the transcription factors Snail (SNAI1), Slug (SNAI2), ZEB1 (ZEB1), and ZEB2 (ZEB2). Expression of AID is induced by inflammatory signals that induce the EMT in nontransformed epithelial cells and in cancer cells. AID regulates expression of master regulators (SNAI1, SNAI2, ZEB1, and ZEB2) in the EMT. Knockdown of AID blocks induction of the EMT and prevents cells from acquiring invasive properties, suppresses expression of several key EMT transcriptional regulators and is associated with increased methylation of CpG islands proximal to the promoters of SNAI1, SNAI2, ZEB1, and ZEB2122. AID-mediated, CpG-methylation dependent mutagenesis is proven to be a common feature of carcinogenesis123. Thus, we have reasons to postulate that re-expression of embryonic factors in cancers as cancer biomarkers might result from epigenetic reprogramming caused by AID/APOBECs, whose expression is induced by proinflammatory factors.

**AID/APOBECs promote tumor heterogeneity**

There are two kinds of tumor heterogeneity: intertumor heterogeneity and intratumor heterogeneity. First, patients with tumors of the same pathologic type show distinct clinical manifestations, including occurrence, metastasis, therapeutic response to chemo- and radiation-therapies, and postoperative prognosis. This heterogeneity is the basis for the development of biomarkers and therapeutic targets that can predict cancer occurrence, metastasis, and therapeutic
response, thus, contributing to personalized medicine. Second, different tumor cells or masses in an individual show significant differences in genomic mutation profile, evolution pathways, and gene expression. This intratumor heterogeneity was discovered and subsequently validated by the application of next-generation sequencing. They found that heterogeneous driver alterations that occurred later in evolution were found in more than 75% of the tumors and were common in PIK3CA and NF1 and in genes that are involved in chromatin modification and DNA damage response and repair. An important emerging mechanism fueling tumor diversity and subclonal evolution is genomic DNA cytosine deamination catalyzed by APOBEC3B and at least one other APOBEC family member. Deregulation of APOBEC3 enzymes by different microenvironment causes a general mutator phenotype that manifests as diverse and heterogeneous tumorsubclones. APOBEC mutational signatures may be enriched in tumorsubclones, indicating APOBECs fuel subclonal expansions and tumor heterogeneity. APOBEC family members might represent a new class of drug target aimed at restricting tumor evolution, adaptation, and even chemo-resistance. APOBEC3B-catalysed deamination provides a chronic source of DNA damage in cancers, thus explaining how some tumors evolve rapidly and manifest heterogeneity. Thus, APOBEC3B-catalysed somatic mutations serve as potential drivers in promoting the formation and progression of tumor heterogeneity.

A small proportion of somatic mutations can lead to advantageous phenotypes that are positively selected during the evolutionary process and thus are called “driver” mutations. The remaining mutations are “passengers” that contribute very little to carcinogenesis. Driver mutations are selected at certain phases of carcinogenesis, but might not be detectable at all stages. At the early stage of carcinogenesis, cells with initial driver mutations can survive and multiply rapidly. However, at the later stages, cells with other driver mutations can gain more advantages in the survival competition. They can replace the cells that have only initial mutations and become the dominant subset. For example, in lung cancer patients who continue to be exposed to tobacco smoking, the signatures of the tobacco-related mutations decline over time, accompanied by an increase in the APOBEC-related mutations. Tracing the positive selection of drivers and the patterns of cancer genomic alteration can help in demonstrating the lineage of the malignancy clones and the major mutagenic factors. Exome-sequencing data from solid tumors and hematologic neoplasms confirmed the clonal heterogeneity of primary tumors and metastases, supporting the evolution model at the genetic level. Thus, the APOBEC-related mutations are more likely to be drivers. Tumors in different microenvironments and at different treatment stages might have distinct mutation spectra, thus demonstrating, within a solid tumor, an obvious heterogeneity that is the result of continuously imbalanced evolution that persists under the selection pressure of the microenvironment. Therapies can also serve as selection pressure, bring their own changes in malignant clones, and that evolution-induced heterogeneity will complicate cancer therapeutic regimes. Cancer therapy should therefore be designed as sequential treatments with the specific purpose of targeting critical pathways during cancer evolutionary process.

Energy metabolism and Cancer Evo-Dev

In the 1920s, Otto Warburg and co-workers showed that tumor tissues metabolize approximately tenfold more glucose to lactate in a given time than normal tissues under aerobic conditions, that is, a preferential use of glycolysis for energy production, even in the presence of oxygen, to support rapid growth of cancer cells, a phenomenon known as the Warburg effect. Warburg hypothesized that this phenomenon occurs due to the malfunction of mitochondria in cancer cells. Up to now, there are two conflicting points of view on effects of mitochondria DNA mutations on the Warburg effect. First, the genetic events that drive aberrant cancer cell proliferation also alter biochemical metabolism, including promoting aerobic glycolysis, but do not typically impair mitochondrial function. Mitochondrial biogenesis and quality control are often upregulated in cancers and mitochondria play a central and multifunctional role in malignant tumor progression. Second, mitochondrial mutations could be the origin of the Warburg phenotype by way of hypoxia-inducible factor activation. Pyruvate kinase M2 (PKM2), an alternatively spliced variant of the pyruvate kinase gene that is preferentially expressed during embryonic development and in cancer cells, alters the final rate-limiting step of glycolysis, resulting in the cancer-specific Warburg effect. PKM2 also mediates EMT via interacting with the transcriptional factor TGF-β-induced factor homeobox 2 to induce the deacetylation of histone H3, thus, resulting in repressed E-cadherin expression. In addition, Warburg effect in tumor-associated macrophages (TAMs) promotes vascular network formation, augments extravasation of tumor cells out of blood vessels, and induces higher levels of EMT at inflammatory foci within the tumor. In microenvironment with both hypoxia and hypoglycemia, stem cell-, angiogenic-, and EMT-biomarkers, as well as glycoprotein-P content and invasiveness of cancer cells are enhanced. Thus, we believe that the Warburg effect promotes the evolutionary process of...
cancer under both hypoxia and hypoglycemia condition. The Warburg effect can provide essential energy for cell survival in hostile microenvironment, furthermore, glycolysis generates the raw material for DNA synthesis of progeny cells.

**ROLES OF CANCER EVO-DEV ON SPECIFIC PROPHYLAXIS AND TARGETED THERAPY OF MALIGNANCIES**

Based on studies of HBV-induced hepatocarcinogenesis, we present the hypothesis of Cancer Evo-Dev to elucidate the critical steps of a common evolutionary and developmental process for most malignancies. The framework of Cancer Evo-Dev can be verified in other cancers such as breast cancer, cervical cancer, head and neck cancer, colorectal cancer, gastric cancer, and lung cancers. Key molecular events occur in the critical steps during Cancer Evo-Dev process can be applied for the occurrence and prognosis prediction and specific prophylaxis of malignant diseases. Furthermore, the core molecules in a functional subnetwork that maintains cancer stemness and promotes Cancer Evo-Dev process can be efficiently targeted by the high-efficiency inhibitors to block corresponding signal pathways, thus providing a powerful treatment strategy for advanced cancers. Thus, Cancer Evo-Dev has three major roles in cancer prophylaxis and treatment: first, to identify what kind of precancerous changes or lesions will develop into cancers; second, to testify what kind of prophylactic option or treatment will reduce the cancer incidence and delay its occurrence; and third, to specifically target key pathways that drive the evolution and development of cancer to reduce morbidity and mortality rates. For the control of HBV-induced HCC, a highly fatal malignancy, active prophylaxis should be of top priority [Figure 5]. Our hypothesis can therefore contribute to the realization of “P4 pattern” medicine (predictive, preventive, personalized, and participatory)\(^2\), therefore promote the prophylaxis and control of cancer.

**DECLARATIONS**

**Authors’ contributions**

G.W. Cao contributed solely to the paper.

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**Conflicts of interest**

There are no conflicts of interest.

**Patient consent**

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Not applicable.

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