研究室リレー紹介　病態制御医学講座 内科学（2）分野

消化器内科

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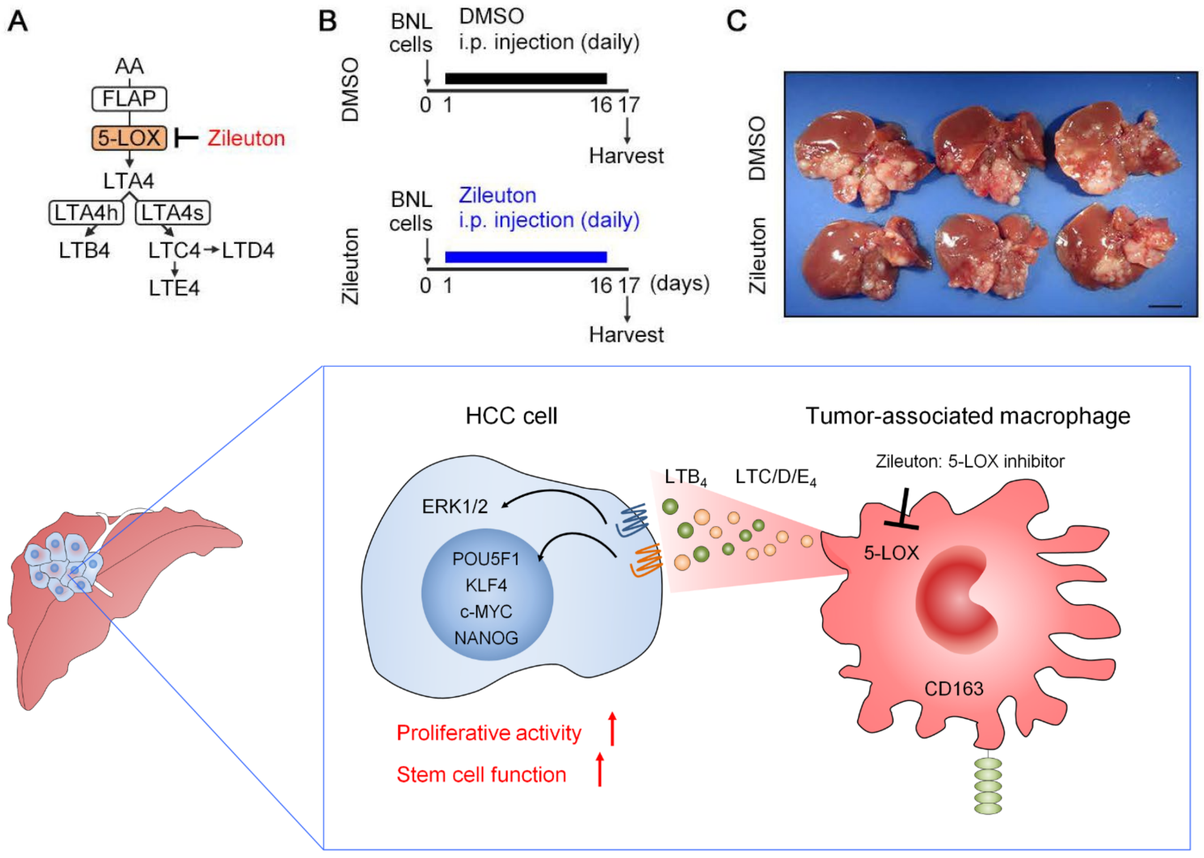
Hepatocellular carcinoma progression promoted by 5-lipoxygenase activity in CD163(+) tumor-associated macrophages

Biomed Pharmacother. 2023 Jun;162:114592.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Arachidonic acid 5-lipoxygenase (5-LOX), an enzyme that synthesizes leukotrienes (LTs), is involved in cancer development including proliferation, invasion, metastasis and drug resistance. However, the functional role of 5-LOX in hepatocellular carcinoma (HCC) remains to be elucidated. In this study, we analyzed the contribution of 5-LOX in HCC progression and investigated the potential of targeted therapy. Analysis of 86 resected HCC specimens and the clinical data of 362 cases of liver cancer from The Cancer Genome Atlas Liver Hepatocellular Carcinoma dataset, showed that 5-LOX expression was associated with postoperative survival. The cancer proliferative and stem cell potential were correlated with the levels of 5-LOX in CD163(+) tumor-associated macrophages (TAMs). In an HCC mouse model, CD163(+) TAMs expressed 5-LOX and produced LTB4 and LTC/D/E4; the 5-LOX inhibitor, zileuton, suppressed HCC progression. LTB4 and LTC/D/E4 promoted cancer proliferation and stem cell capacity via phosphorylation of extracellular signal-regulated kinase 1/2 and stem cell-associated genes. Taken together, we identified a novel mechanism of HCC progression in which CD163(+) TAMs express 5-LOX and produce LTB4 and LTC/D/E4, thereby enhancing the proliferative and stem cell potential of HCC cells. Furthermore, inhibition of 5-LOX activity regulates HCC progression, suggesting it has potential as a new therapeutic target.

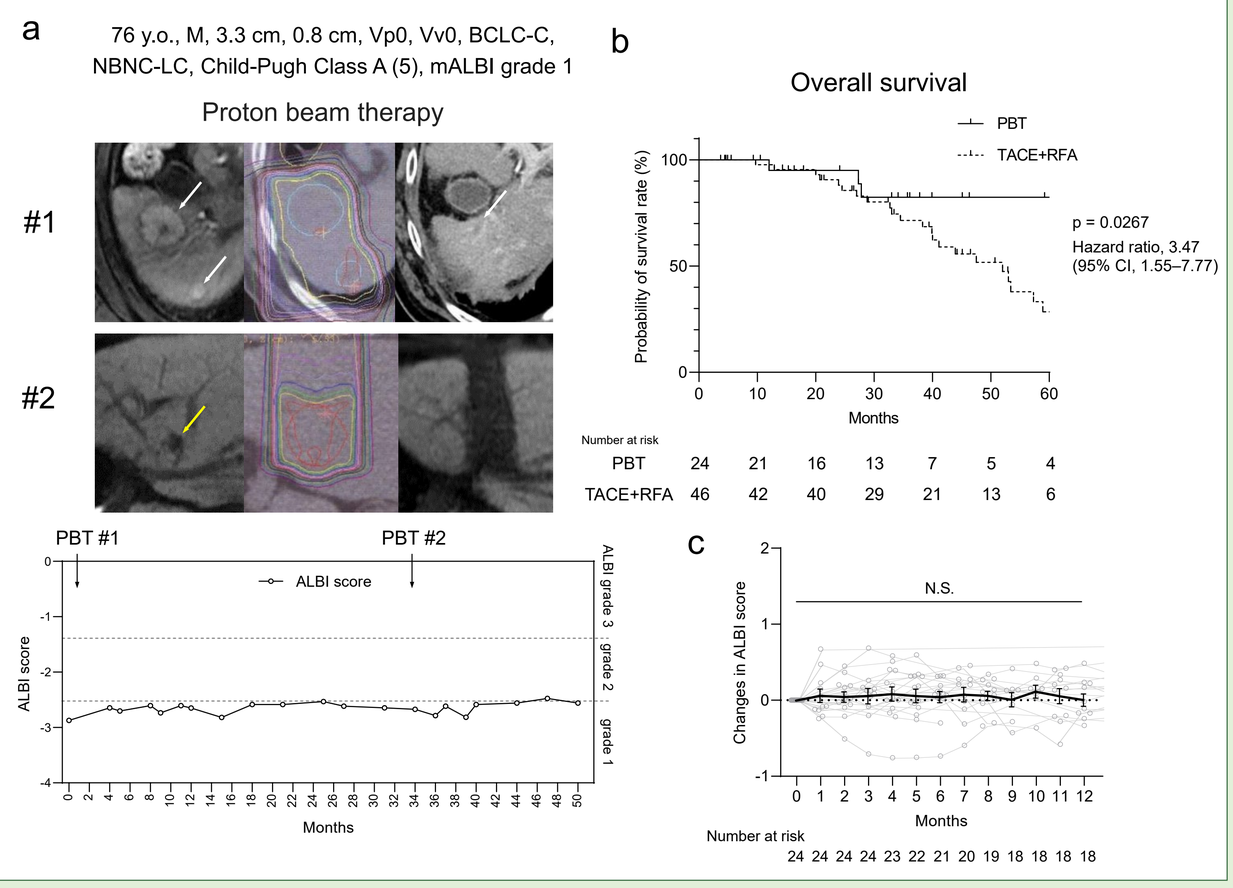


Longer Survival and Preserved Liver Function after Proton Beam Therapy for Patients with Unresectable Hepatocellular Carcinoma

Curr Oncol. 2023 Mar 30;30(4):3915-3926.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Background: Proton beam therapy (PBT) has been recently reported to achieve excellent tumor control with minimal toxicity in patients with unresectable hepatocellular carcinoma (HCC). Radiofrequency ablation (RFA) combined with transcatheter arterial chemoembolization (TACE) was investigated for larger HCC. This study was designed to evaluate the therapeutic effect of PBT on unresectable HCC in comparison with TACE combined with RFA.
Methods: We retrospectively analyzed 70 patients with HCC which was difficult to control by surgical resection or RFA monotherapy, 24 patients treated with PBT and 46 patients with TACE plus RFA. The therapeutic effects were assessed as local progression-free survival (PFS) and overall survival (OS).
Results: The local PFS was more than 65% in 60 months for PBT and TACE plus RFA. The patients treated with PBT showed 82% OS at 60 months post-treatment. In contrast, those treated with TACE plus RFA showed 28% OS. When comparing the changes of ALBI scores in patients with different severities of chronic liver disease, the scores of PBT-treated patients were maintained at the baseline; however, those of TACE plus RFA-treated patients worsened after the treatments.
Conclusions: The results indicated that PBT may show better benefits than TACE plus RFA therapy in terms of OS in patients with unresectable HCC by sparing the non-tumor liver tissues.

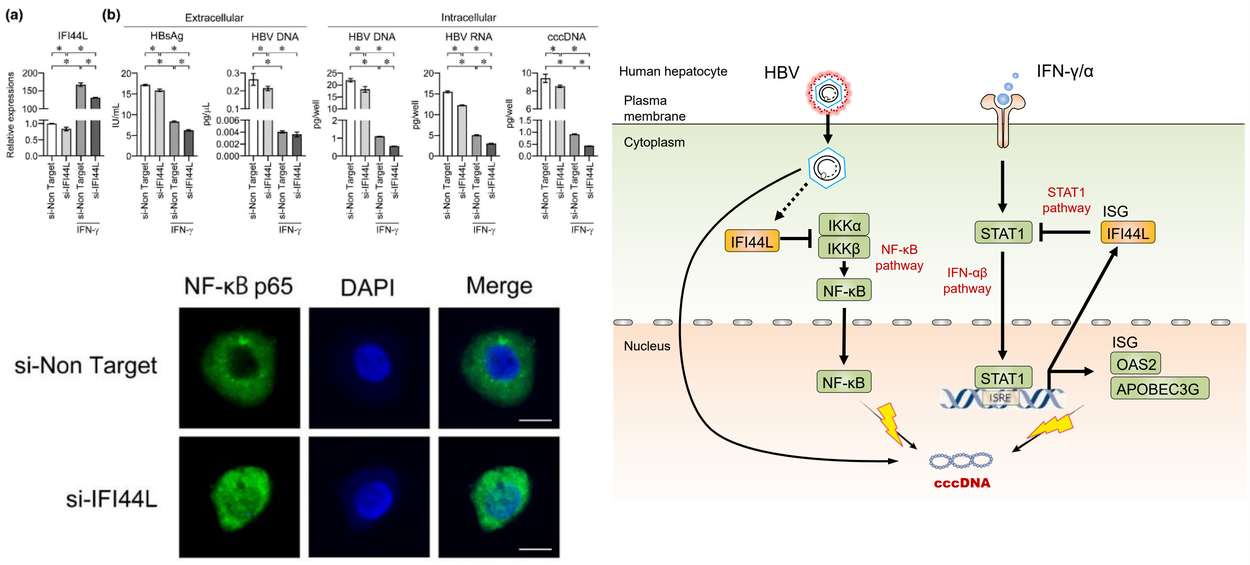


Regulatory function of interferon-inducible 44-like for hepatitis B virus covalently closed circular DNA in primary human hepatocytes

Hepatol Res. 2022 Feb;52(2):141-152.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Aim: Curing hepatitis B virus (HBV) infection requires elimination of covalently closed circular DNA (cccDNA). Interferon (IFN)-γ has noncytolytic antiviral potential; however, elimination of cccDNA could not be achieved. To enhance the regulatory effect, we comprehensively analyzed the host factors associated with cccDNA amplification and IFN-γ and IFN-α effects using an in vitro HBV infection system showing various transcription levels.
Methods: Primary human hepatocytes were infected with HBV using genomic plasmids carrying the basic core promoter mutation A1762T/G1764A and/or the precore mutation G1896A and treated with IFN-γ and IFN-α. Comprehensive and functional studies involving microarray and small interfering RNA analysis revealed the host factors related to cccDNA regulation.
Results: The HBV infection system reproduced the HBV life cycle and showed various propagation levels. Microarray analysis revealed 53 genes correlated with the cccDNA levels. Of the 53 genes, expression of IFN-induced protein 44-like (IFI44L) was significantly upregulated by IFN-γ and IFN-α. The anti-HBV effect of IFI44L is exerted regardless of IFN-γ or IFN-α by inhibiting the activation of nuclear factor-κB and signal transducer and activator of transcription 1 pathways.
Conclusions: Using the in vitro HBV infection system, an IFN-inducible molecule, IFI44L, associated with cccDNA amplification, was identified. These results suggest an innovative molecular strategy for the regulation of HBV cccDNA by controlling a novel host factor, IFI44L.

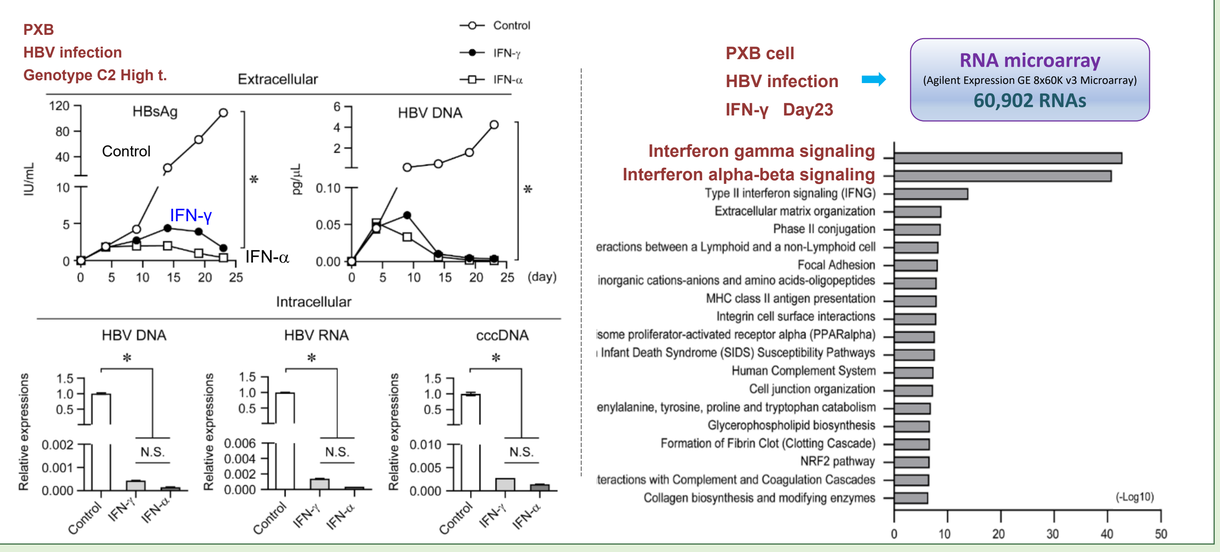


Molecular signature of hepatitis B virus regulation by interferon-γ in primary human hepatocytes

Hepatol Res. 2020 Mar;50(3):292-302.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Aim: A complete cure for chronic hepatitis B virus (HBV) infection requires elimination of covalently closed circular DNA; however, this remains to be clinically achieved. Interferon (IFN)-γ, a type II IFN, is produced by intrahepatic cytotoxic T lymphocytes and has non-cytolytic antiviral potential. However, the mechanism by which IFN-γ regulates HBV infection has not been fully elucidated. Thus, we developed an in vitro HBV infection assay system and analyzed the molecular signature of HBV regulation by IFN-γ.
Methods: The in vitro HBV infection assay system was established in primary human hepatocytes infected with HBV derived from the plasmid containing 1.3-mer HBV genome, and treated with IFN-γ. The antiviral effects and signaling pathways of IFN-γ were examined using microarray, and assessed by siRNA knockdown experiments of the related genes.
Results: IFN-γ treatment suppressed both HBV propagation and transcription as efficiently as IFN-α. Microarray analysis showed that IFN-γ stimulation induced the activation of both IFN-γ and IFN-α signaling, regulating HBV covalently closed circular DNA. HBV production was decreased by IFN-γ through Janus kinase/signal transducer and activator of transcription signaling and interferon-stimulated genes, such as 2'-5'-oligoadenylate synthase 2 and apolipoprotein B mRNA editing enzyme catalytic subunit 3G.
Conclusions: IFN-γ can suppress HBV propagation and transcription in hepatocytes by activating specific intracellular signaling pathways in hepatocytes, and suggests the future application of these particular signaling pathways or genes for the complete elimination of HBV.

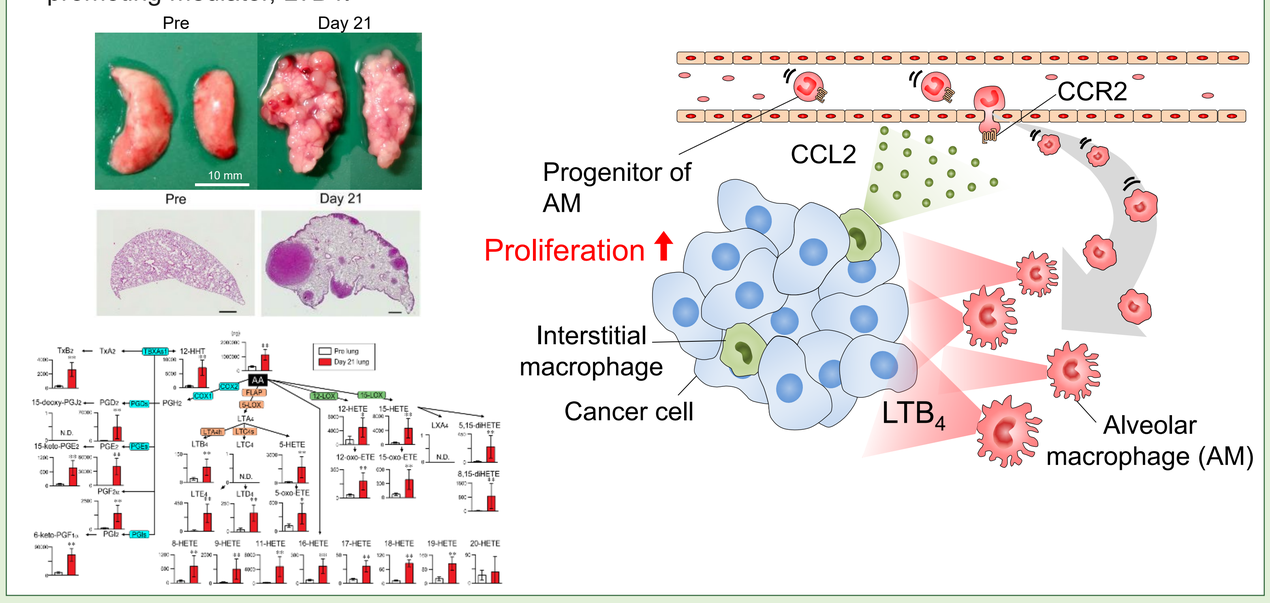


Alveolar Macrophages Drive Hepatocellular Carcinoma Lung Metastasis by Generating Leukotriene B4

J Immunol. 2018 Mar 1;200(5):1839-1852.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Macrophages in lungs can be classified into two subpopulations, alveolar macrophages (AMs) and interstitial macrophages (IMs), which reside in the alveolar and interstitial spaces, respectively. Accumulating evidence indicates the involvement of IMs in lung metastasis, but the roles of AMs in lung metastasis still remain elusive. An i.v. injection of a mouse hepatocellular carcinoma (HCC) cell line, BNL, caused lung metastasis foci with infiltration of AMs and IMs. Comprehensive determination of arachidonic acid metabolite levels revealed increases in leukotrienes and PGs in lungs in this metastasis model. A 5-lipoxygenase (LOX) inhibitor but not a cyclooxygenase inhibitor reduced the numbers of metastatic foci, particularly those of a larger size. A major 5-LOX metabolite, LTB4, augmented in vitro cell proliferation of human HCC cell lines as well as BNL cells. Moreover, in this lung metastasis course, AMs exhibited higher expression levels of the 5-LOX and LTB4 than IMs. Consistently, 5-LOX-expressing AMs increased in the lungs of human HCC patients with lung metastasis, compared with those without lung metastasis. Furthermore, intratracheal clodronate liposome injection selectively depleted AMs but not IMs, together with reduced LTB4 content and metastatic foci numbers in this lung metastasis process. Finally, IMs in mouse metastatic foci produced CCL2, thereby recruiting blood-borne, CCR2-expressing AMs into lungs. Thus, AMs can be recruited under the guidance of IM-derived CCL2 into metastatic lungs and can eventually contribute to the progression of lung metastasis by providing a potent arachidonic acid-derived tumor growth promoting mediator, LTB4.

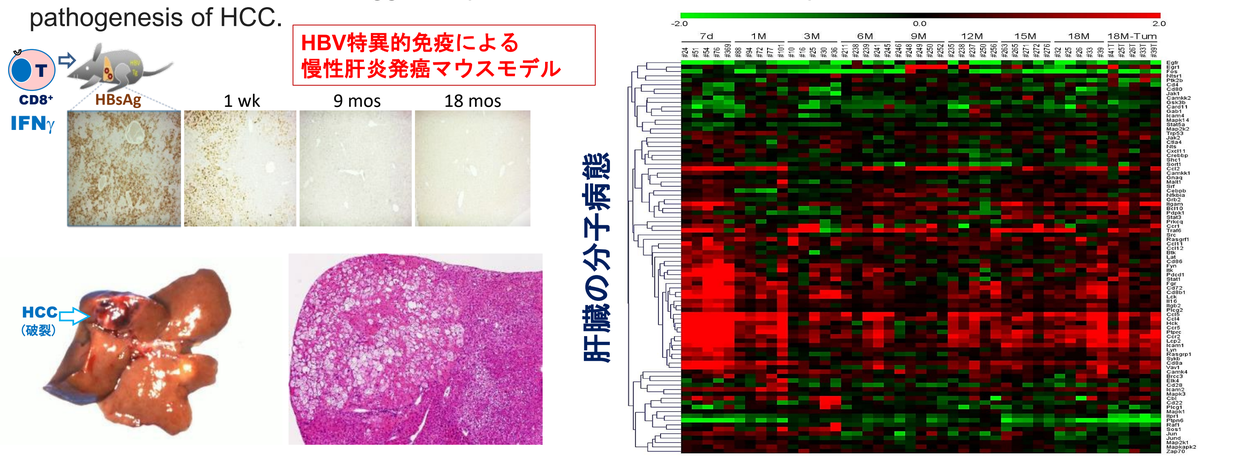


Gene expression profiling of hepatocarcinogenesis in a mouse model of chronic hepatitis B

PLoS One. 2017 Oct 2;12(10):e0185442.

Takuto Nosaka, Yasunari Nakamoto *et al.*

Background: Hepatocellular carcinoma (HCC) is a common complication of chronic viral hepatitis. In support of this notion, we have reported that hepatitis B surface antigen (HBsAg)-specific CD8+ T lymphocytes critically contribute to inducing chronic liver cell injury that exerts high carcinogenic potential in a hepatitis B virus (HBV) transgenic mouse model. The dynamics of the molecular signatures responsible for hepatocellular carcinogenesis are not fully understood. The current study was designed to determine the serial changes in gene expression profiles in a model of chronic immune-mediated hepatitis.
Methods: Three-month-old HBV transgenic mice were immunologically reconstituted with bone marrow cells and splenocytes from syngeneic nontransgenic donors. Liver tissues were obtained every three months until 18 months at which time all mice developed multiple liver tumors. Nitrative DNA lesions and hepatocyte turnover were assessed immunohistochemically. Gene expression profiles were generated by extracting total RNA from the tissues and analyzing by microarray.
Results: The nitrative DNA lesions and the regenerative proliferation of hepatocytes were increased during the progression of chronic liver disease. In a gene expression profile analysis of liver samples, the chemokine- and T cell receptor (TCR)-mediated pathways were enhanced during chronic hepatitis, and the EGF- and VEGF-mediated pathways were induced in HCC. Among these molecules, the protein levels of STAT3 were greatly enhanced in all hepatocyte nuclei and further elevated in the cytoplasm in HCC tissue samples at 18 months, and the levels of phosphorylated TP53 (p-p53-Ser 6 and -Ser 15) were increased in liver tissues.
Conclusions: HBV-specific immune responses caused unique molecular signatures in the liver tissues of chronic hepatitis and triggered subsequent carcinogenic gene expression profiles in a mouse model. The results suggest a plausible molecular basis responsible for HBV-induced immune pathogenesis of HCC.



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http://www-n.med.u-fukui.ac.jp/laboratory/second-internal/

内科学(２)分野の紹介動画をご覧ください。

https://www.youtube.com/watch?v=yCdzmk8npIw