of organ-specific genomic changes in oncogenic pathways are not well known. Interestingly, organ-specific changes in mutational profiles were observed in the epithelial tissues of the AID transgenic mice. Indeed, the *c-Myc* gene was mutated frequently in noncancerous tissue of the lung, whereas *K-ras* gene mutations frequently were detectable in gastric cancer developed in AID transgenic mice.[84] Thus, the organ-specific differences in the mutational profiles in AID transgenic mice suggest the possibility that the target preference of AID-induced mutagenesis in different tissues might contribute to the diversity of tissue-specific oncogenic pathways in various epithelial organs.

In vitro analyses using human cultured cells with constitutive AID expression revealed that *TP53* mutations were induced frequently by AID genotoxic activity in hepatocytes, and gastric, colonic, and bile duct epithelial cells.[77,79–81] Similar to the *TP53* gene, the cyclin-dependent kinase inhibitor (*CDKN*)-*2B-CDKN2A* locus was identified as a target for AID-mediated genotoxic activity. The *CDKN2B-CDKN2A* locus encodes the potent suppressor proteins $p16^{INK4a}$, $p15^{INK4b}$, and $p14^{ARF}$, which regulate the activities of the retinoblastoma protein and the TP53 transcription factor. Aberrant AID expression preferentially induces somatic mutations at the *CDKN2B-CDKN2A* locus in gastric epithelial cells and biliary cells.[81,87] Moreover, comparative genomic hybridization analysis clearly showed that constitutive AID activation in cultured gastric epithelial cells caused submicroscopic deletions as represented by copy number losses of various chromosomal loci, especially at the *CDKN2B-CDKN2A* locus at 9p21. Copy number reduction of *Cdkn2b-Cdkn2a* also was seen in the gastric mucosa of AID transgenic mice.[87] In agreement with the preferential deletions at the *CDKN2B-CDKN2A* locus in gastric epithelial cells by AID introduction, AID expression was required for inducing DNA single-strand breaks in the *CDKN2B* gene in leukemia cells,[88] and, furthermore, the deletion of the *CDKN2B-CDKN2A* locus frequently is detectable in AID-expressing lymphoid blast crisis leukemia cells.[75] These findings suggest that AID can induce both mutations and deletions at the same gene locus, and, moreover, that the representative tumor-suppressor genes, *TP53* and *CDKN2B-CDKN2A*, may be common targets for AID-mediated genotoxic effects in various human tissues in the setting of inflammation.

Finally, a recent finding that a deficiency of endogenous AID reduced the incidence of both accumulation of somatic mutations in the *Trp53* gene and the development of colitis-associated colorectal cancers further supports the critical role of AID in inflammation-associated cancer development via its ability to induce genetic alterations in tumor-related genes.[89]

Inflammation and Epigenetic Modulation

Epigenetic modifications are DNA-associated modifications that are inherited upon somatic cell replication, which include DNA methylation and histone modifications.[90] Coordinated changes of epigenetic modifications control development and tissue differentiation, and erasure of epigenetic modifications is involved in reprogramming. In somatic cells, DNA methylation is present in repetitive elements, CpG-sparse regions, and in a very limited number of CpG islands.[91,92] DNA methylation of a CpG island in a promoter region causes silencing of its downstream gene, whether it is a protein-coding gene or a microRNA (miRNA) gene, by forming nucleosomes and thus possibly blocking access of RNA polymerase II to the promoter.[93,94] In contrast, DNA methylation of a gene body often is associated with increased gene expression.[91,95]

Histone modifications denote chemical modifications, such as acetylation, methylation, and ubiquitination of lysine and arginine residues of histones, mainly H3 and H4, but also H2A and H2B.[93] Specific histone modifications, such as acetylation of histones H3 and H4 (H3Ac and H4Ac) and trimethylation of lysine 4 of histone H3 (H3K4me3), are associated with active gene transcription. In contrast, dimethylation and trimethylation of H3 lysine 9 (H3K9me2 and H3K9me3) and trimethylation of H3 lysine 27 (H3K27me3) are associated with gene repression. H3K9me2 represses gene transcription in concert with DNA methylation, whereas H3K27me3 works independently of DNA methylation.[96] Trimethylation of H3 lysine 36 (H3K36me3) is considered to mark exonic regions of active genes. However, the mechanisms of how histone modifications are inherited upon somatic cell replication remains unclear.[97]

In cancer cells, the presence of regional hypermethylation and global hypomethylation has been described.[98,99] Regional hypermethylation refers to aberrant DNA methylation of promoter CpG islands physiologically kept unmethylated.[95,100] If aberrant methylation is induced in a promoter CpG island it consistently induces silencing of its downstream gene.[90] Many tumor-suppressor genes that have promoter CpG islands, such as *CDKN2A*, mutL homolog 1 (*MLH1*), cadherin-1, and RAS-association domain family 1, isoform A, can be inactivated permanently by aberrant DNA methylation as drivers, which have significant roles in cancer development. At the same time, most of the aberrant DNA methylation of promoter CpG islands are considered to be passengers that play no role in carcinogenesis.[14] Several hundreds to thousands of promoter CpG islands are methylated aberrantly in a cancer, and the number is too large for all of them to be drivers. Moreover, most of the genes methylated in cancers are not expressed in normal tissues,[101,102] and such genes are considered not to be involved in carcinogenesis. Global hypomethylation was shown to be causally involved in carcinogenesis by inducing genomic instability.[103] In addition, induction of H3K27me3 is considered to be an alternative mechanism to induce gene silencing,[96] and aberrant H3K27me3 was observed in promoter regions consisting of 200–600 genes.[96,104] Again, the number is very large, and most are expected to be passengers.

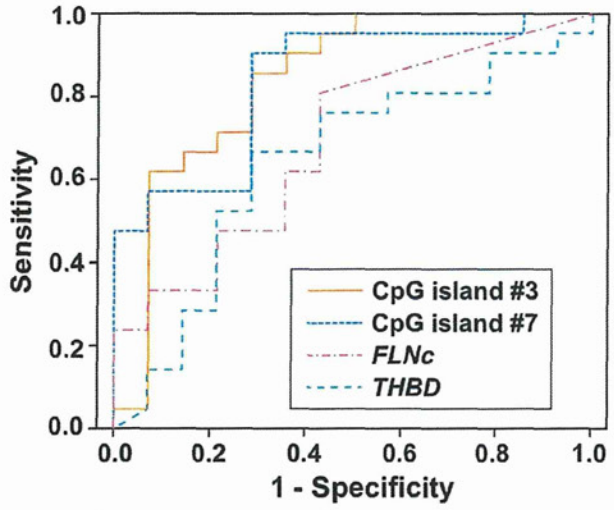

Figure 4. The degree of epigenetic field defects can be assessed using methylation levels of appropriate marker CpG islands, mostly passengers. Receiver-operating characteristic curves were drawn to distinguish gastric mucosae of gastric cancer patients and those of healthy individuals with past infections by *H pylori*. The receiver-operating characteristic curves of newly isolated methylation risk markers, CpG islands #3 and #7, had a much larger area under the curve values than those of 2 previously isolated markers, filamin C (FLNc) and thrombomodulin (THBD), reaching 0.78–0.84. Modified from Nanjo et al.[169]

As inducers of aberrant DNA methylation, aging was first indicated,[105] and chronic inflammation then was suggested by the presence of aberrant DNA methylation of specific tumor-suppressor genes in noncancerous colonic mucosae of patients with IBD.[106,107] Aberrant DNA methylation was present more frequently in liver tissues of patients with HCC than in those with metastatic liver tumors.[108] By measuring methylation levels of passenger genes in gastric mucosae of *H pylori*–infected individuals, a very close association between *H pylori* infection and high methylation levels in gastric mucosa was shown.[15] Aberrant DNA methylation is particularly prominent in chronic inflammation-associated cancers, such as gastric cancer, HCCs, colitic cancer, cholangiocarcinoma, Barrett's cancer, and pancreatic cancer.[13] These findings strongly indicated that the major inducer of aberrant DNA methylation is chronic inflammation.

Levels of aberrant DNA methylation accumulated in normal-appearing tissues correlate with the risk of gastric, colon, breast, and renal cancers.[15,109–112] Such accumulation mainly involves passenger genes and driver genes to some extent, and is considered to form an epigenetic field for cancerization (epigenetic field defect) (Figure 4).[113] Chronic inflammation-associated cancers are known to show multiple events, which can be explained by the presence of a field defect in normal-appearing tissues. Along with the accumulation of genetic alterations, an epigenetic field defect is deeply involved in the development of inflammation-associated cancers. The degree of epigenetic field defects can be measured easily using methylation levels of marker genes,[114] which are passenger genes in most cases and show relatively high methylation levels in predisposed tissues.[113]

Mechanistic studies, including cause and effect of accumulated aberrant DNA methylation and chronic inflammation, were conducted using animal models. When *H pylori*–induced inflammation was suppressed by cyclosporine A in Mongolian gerbils, induction of aberrant DNA methylation markedly was suppressed, although the number of *H pylori* in gastric mucosae was unaffected.[16] This indicated that inflammation, not *H pylori* itself, is critical for induction of aberrant DNA methylation. Expression analysis of inflammation-related genes showed that expression levels of *Il-1b*, *Nos*, *Tnf*, and chemokine (C-X-C motif) ligand 2 correlated with methylation levels in gastric mucosae. *H pylori*–induced inflammation was capable of inducing aberrant DNA methylation, but not repeated induction of acute inflammation by ethanol or a high sodium concentration.[115] *Il-1β*, *Nos2*, and *Tnf* were specifically up-regulated by the *H pylori*–induced inflammation. Notably, in human beings, a polymorphism of the *IL-1B* promoter was associated with not only gastric cancer susceptibility,[35] but also the presence of the CpG island methylation phenotype in gastric cancers.[116]

Another animal model for methylation induction by chronic inflammation is mouse colitis induced by administration of dextran sulfate sodium (DSS).[117] Aberrant DNA methylation of multiple genes occurred in DSS-induced colitis mucosae before induction of colon tumors, showing an epigenetic field.[118] The induction of aberrant DNA methylation was unaffected even in severe combined immunodeficiency mice that lacked T and B cells, suggesting that infiltrated macrophages might be critical for methylation induction. Gene expression analysis in colonic mucosae in wild-type and severe combined immunodeficiency mice showed that expression levels of *Il-1b*, *Nos*, and *Ifn-g* were associated with methylation induction in colonic mucosae. Taken together with the finding in the *H pylori*–infected gerbils, infiltration of macrophages and resulting secretion of Il-1β and Tnf-α, as well as production of active oxygen species, are believed to be involved in induction of aberrant DNA methylation in epithelial cells (Figure 1).

Several in vitro studies have been conducted to examine inflammatory signals that lead to methylation induction in target cells. Treatment of insulinoma or blood cells with IL-1β or a NO donor induced methylation of endogenous genes by increasing activity of DNA methyltransferase(s) (DNMTs).[119] IL-6 induces DNMT1 transcription by increasing its promoter activity and suppressing microRNA (miR)-148a and miR-152, both of which target DNMT1.[120,121] Although some studies suggested that DNA methylation is induced by IL-1β or IL-6, the changes were marginal, possibly because identification of appropriate target CpG islands was difficult and the levels of increase were too small to be detected by ordinary methods. Prostaglandin E2 treatment of cancer cell lines increased DNMT1 and DNMT3B expression, and induced

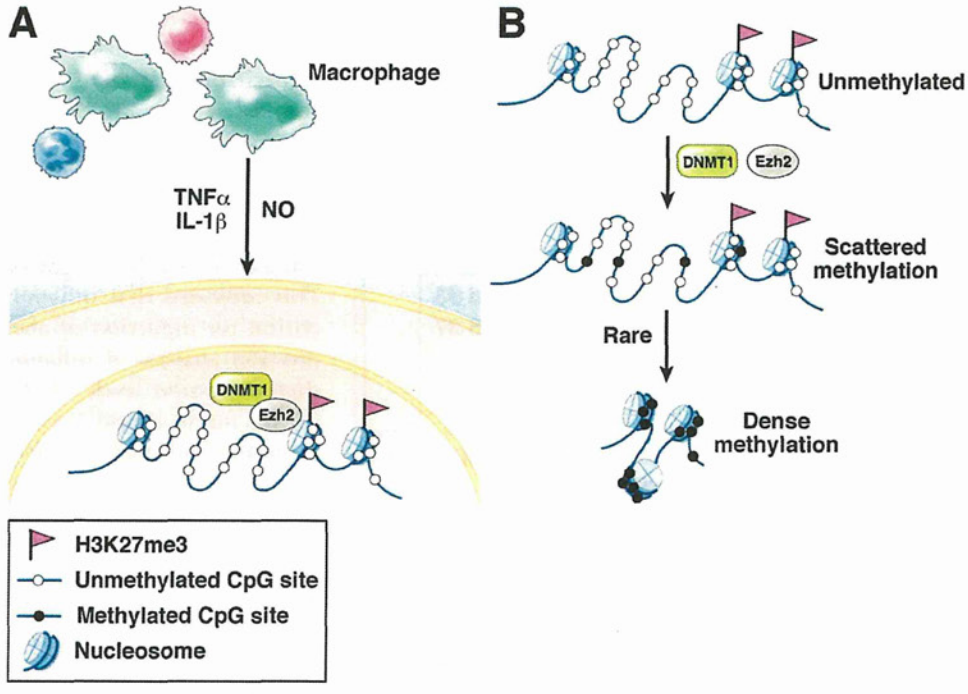

Figure 5. Current model of aberrant DNA methylation induction by chronic inflammation. (*A*) Cytokines, such as IL-1 and TNF-a from macrophages, and oxidative stress, such as NO, are associated with methylation induction in epithelial cells. EZH2 and DNMT1 are reported to be recruited to a promoter CpG island of a damaged gene, and mark it with a flag of H3K27me3. (*B*) Scattered methylation, introduced by DNMT1, leads to dense methylation although the frequency is low.

DNA methylation of specific genes, which also was observed in vivo.[122]

In contrast to in vitro studies, mRNA expression levels of Dnmt1, Dnmt3A, and Dnmt3B were not increased in vivo, such as colonic mucosae with DSS-induced colitis,[16] and human gastric tissues with *H pylori* infection.[123] In line with these in vivo findings, O'Hagan et al[124] recently showed in vitro that oxidative damage recruits complexes containing DNMTs, a histone deacetylase (sirtuin 1), and histone methyltransferase (enhancer of zeste homolog 2 [EZH2]) to damaged chromatin, and induces DNA methylation. They also showed that in Apc^{Min} mice infected with an inflammation-inducing bacterium, Dnmt1 and Ezh2 are recruited to promoter CpG islands of untranscribed or minimally transcribed genes. Promoter CpG islands with H3K27 me3 and without RNA polymerase II are susceptible to DNA methylation induction.[101,102]

Taken together, we can hypothesize a model for aberrant DNA methylation induction in vivo (Figure 5). Inflammatory signals mainly from macrophages, such as IL-1β, TNF-α, and IL-6, and oxidative stress, possibly produced by NO synthase, are likely to recruit a complex with DNMT1 and EZH2 to promoter CpG islands with H3K27 me3 flag and without protection by RNA polymerase II. Because DNA methylation is harmful to a gene, aberrant DNA methylation is likely to be induced only rarely and at scattered CpG sites within a CpG island (seeds of methylation).[123] Most seeds of methylation are erased during cell replication, but can lead to dense methylation of a CpG island at very low frequencies.[125,126] If such dense methylation is induced in a promoter CpG island of a tumor-suppressor gene, the tissue becomes predisposed to carcinogenesis, and forms an epigenetic field defect.

In addition to aberrant DNA methylation of promoter CpG islands, cancer cells are characterized by global DNA hypomethylation as well as aberrant hypomethylation of oncogenes.[99,127] Gastric mucosa infected by *H pylori* displays global hypomethylation.[128] In this regard, it is interesting to note that AID recently was shown to be involved in active DNA demethylation during fetal development.[129] Mechanistically, AID deaminates 5-methyl cytosine to yield T. This T subsequently would be removed by either of the T:G mismatch-specific glycosylases, thymidine DNA glycosylase, or methyl-CpG binding domain protein 4. The resulting abasic site then would be replaced by an unmethylated C via base excision repair processes, resulting in DNA demethylation. Notably, AID participates in active demethylation by 5-methyl cytosine hydroxylase, ten-eleven translocation 1, and subsequent gene expression in the dentate gyrus of adult mouse brain.[130] Thus, whether AID is involved in DNA demethylation during cancer development is an interesting topic for future studies.[131] The fact that AID targets the chromatin marked by H3K4 me3 histone modification,[132] in contrast to preferential DNA methylation at promoter CpG islands with H3K27 me3 histone modification,[101,102] might suggest opposing mechanisms for induction of DNA methylation and demethylation.

Inflammation and miRNA Modulation

miRNAs are short noncoding RNAs that regulate the expression of many target genes post-transcriptionally, and thus are involved in a variety of cellular functions. Recent studies have revealed that miRNAs have important roles in cancer development as either oncogenes or tumor-suppressor genes by regulating various cancer-related proteins or mRNA expressions.[133,134] In-

deed, cancer cells are associated with dysregulation of many miRNA expressions, which occurs through a variety of mechanisms, such as genetic changes, epigenetic regulation, or altered expression of transcription factors.[135] On the other hand, miRNA expression also is altered in inflammatory conditions, and such alterations in miRNA expression appear to play roles not only in controlling chronic inflammation, but also in promoting cancer development.[136,137] Many of the changes in miRNA expressions observed in inflammatory tissues are derived from immune cells that may participate in hematopoietic tumorigenesis.[138] However, recent reports have shown that inflammation also induces changes in cancer-related miRNAs in epithelial cells, suggesting a direct link between alteration of miRNA expressions and inflammation-associated cancer development.[139,140]

miRNA expressions in epithelial cells can be altered during inflammation through various mechanisms such as NF-κB activation by Toll-like receptors or cytokine stimulation and STAT3 phosphorylation by IL-6 or other cytokines.[139-143] Among those, several miRNAs are identified as tumor-suppressor miRNAs. *miR-7* targets not only epidermal growth factor receptor (*Egfr*) but also latrophilin 2, brain abundant, membrane-attached signal protein 1, and musculoaponeurotic fibrosarcoma oncogene homolog, and thus is considered to be a tumor-suppressor miRNA.[142] In a mouse model of inflammation-associated cancer development, expression of *miR-7* was shown to be inhibited by activated macrophages in *Helicobacter*-infected gastritis mucosa and was shown to be involved in gastric cancer development, although it was increased in germ-free conditions.[142] Lethal-7 (*Let-7*), consisting of 12 members, targets the *RAS* family and *c-MYC*,[144,145] and genomic locations of *let-7* family members frequently are deleted in colon cancers and other solid cancers.[146] NF-κB activation enhances *Lin28B* transcription, which causes posttranscriptional inhibition of *let7* family member expression, and *let-7* directly inhibits IL-6 expression, a cytokine often produced in cancer cells. Thus, reduction of *let-7* expression by NF-κB activation appears to play a role in a positive feedback loop for NF-κB activation through an increase of IL-6 in cancer cells.[147]

miR-155, a possible oncogenic miRNA, is involved in blood cell maturation, immune responses, and autoimmune disorders, and high expression of *miR-155* is associated with the development of myeloproliferative disorders.[148] Recent studies have revealed a direct link between increase of *miR-155* and tumor formation and development in gastric and colon cancers.[148,149] *miR-155* expression is induced by NF-κB, interferon-β, and TLR stimulation,[150] and thus enhanced by *H pylori* and lipopolysaccharide (LPS) treatment.[151] Recently, Tilli et al[143] reported that TNF-α/LPS stimulation enhances *miR-155* expression in association with an increased mutation rate. They also showed that *miR-155* targets mitosis inhibitor protein kinase 1, which blocks cell-cycle progression, and therefore reasoned that reduction of mitosis inhibitor protein kinase by *miR-155* allowed cell division to continue even in the presence of DNA damage, leading to enhanced mutation induction. In another study, they also showed that *miR-155* promotes gene mutations by down-regulating the core mismatch repair proteins, hMSH2, hMSH6, and hMLH1.[152] Of particular interest are the recent reports showing that *miR-155* negatively regulates AID in B cells. Teng et al[153] showed that *miR-155* is up-regulated in B cells undergoing class-switch recombination, and regulates the germinal center reaction by modulating AID. Moreover, *miR-155* has been suggested to inhibit *MYC-IGH* translocation by reducing AID mRNA and protein in B cells.[154] Thus, although an inhibitory effect of *miR-155* on AID has not been examined in non–B cells, *miR-155* also may have a tumor-suppressor function in epithelial cells by inhibiting AID production.

A miRNA expression pattern distinct from normal colonic mucosa has been found in the colonic mucosa and in colitic tumor of patients with IBD as well as mice with colonic inflammation, including up-regulation of *miR-21* and *miR-3*.[155] *miR-21* is one of the most highly expressed miRNAs in colonic tissues of patients with ulcerative colitis,[155] and its expression is enhanced by LPS and IL-6 through STAT3 activation, targeting key regulators of cell proliferation and apoptosis such as phosphatase and tensin homolog and programmed cell death 4.[156] Olaru et al[157] recently showed that in colitic cancer development *miR-31* expression increases in a stepwise fashion from IBD to cancer, and that *miR-31* directly targets regulating factor inhibiting hypoxia-inducible factor 1, decreasing its repressor activity for hypoxia-inducible factor 1.

It is now evident that miRNAs exert various functions in inflammation-associated cancer development. However, alterations of miRNA expression observed in inflammatory tissues occur in both immune cells and epithelial cells. Accordingly, it is important to dissect miRNA changes in the 2 cell types because the patterns of the miRNA changes are different between immune cells and epithelial cells. Further elucidation of the changes of miRNA expression, particularly in epithelial cells, will facilitate our understanding of the role of tumor-related miRNAs in inflammation-associated cancer development.

Application to Cancer Prevention, Diagnostics, and Therapeutics

To prevent inflammation-associated cancer development, it is crucial to cure or control inflammation. Indeed, it has been shown repeatedly that long-term therapy with anti-inflammatory drugs resulted in fewer appearances of tumors.[158] The best way to control chronic inflammation is, of course, to eliminate causative infections. In other cases unrelated to infection such as IBD and PSC, one approach is to block the action of key regulators of inflammation. In this regard, NF-κB or STAT3, and their activators TNF-α or IL-6, respectively, may be good targets for suppressing the inflammatory response. However, because treatment usually needs to be continued for long periods to control chronic inflamma-

tion, agents without serious side effects with lower costs should be developed. For this purpose, many natural agents derived from vegetables, fruits, spices, and their components have been tested. Among them, curcumin, derived from yellow spice turmeric (Curcuma longa) has been used for centuries, and has been shown to suppress NF-κB- as well as STAT3-regulated inflammation,[159] and thus can be administered safely over the long term.[160] Indeed, a recent study showed that curcumin reduced TNF-α expression, prevented cancer-associated weight loss, and induced apoptosis of tumors in patients with colorectal cancer.[161] Resveratrol, a natural polyphenolic, nonflavonoid antioxidant found in grapes and other berries has been shown to have generalized inhibitory effects on inflammation-related molecules such as NF-κB, COX-2, and tyrosine kinases.[162] Recently, resveratrol was found to alter the expression of many tumor-related miRNAs.[163] Similar types of agents may have the potential to both prevent and treat cancers.[164]

In contrast to controlling inflammatory mediators, blocking genetic modulation appears to be difficult. One might consider inhibiting AID. However, because AID plays a critical role in immunoglobulin maturation in B cells, specific targeting for AID in the epithelial cells without affecting AID in B cells is critical. Control of epigenetic modulation can be considered from 2 aspects: suppression of methylation induction and reversal of induced methylation. Because induction of methylation is not essential in adult somatic cells, control of this process is a promising approach to prevent chronic inflammation-associated cancers. On the other hand, reversal of aberrant DNA methylation is an attractive idea to repair an epigenetic field defect, but targeting only aberrant DNA methylation without affecting physiological DNA methylation is currently very difficult.

H pylori eradication ameliorates chronic inflammation, and reduces the risk for gastric cancer. However, it is apparent that eradication cannot completely resolve chronic inflammation because some patients develop gastric cancer even after successful eradication.[165] Likewise, some patients with chronic hepatitis or cirrhosis as a result of HCV infection also develop HCC after obtaining a sustained virologic response.[166] As such, when inflammation is not appropriately controlled or even when inflammation is resolved after long-standing inflammation, accurate prediction for the risk of developing cancers in the inflammatory tissues becomes important. As was discussed, carcinogenesis is characterized by a stepwise accumulation of both genetic and epigenetic changes. Importantly, previous data suggested that the extent of those genetic and epigenetic modulations is paralleled with the duration or severity of inflammation,[15,167] and the degree of epigenetic field defect can be measured relatively easily and accurately. Thus, both qualitative and quantitative detection of these genetic and epigenetic changes in inflammatory tissues or tissues previously exposed to inflammation may provide a good risk marker for inflammation-associated cancer development. Indeed, epigenetic risk markers that can differentiate gastric mucosae of cancer patients from those of healthy individuals with odds ratios between 12.7 and 36.0 have been isolated,[168,169] and a prospective study is now being conducted.

Conclusions

Many cancers in digestive organs develop in the background of chronic inflammation. During chronic inflammation, a variety of mediators for inflammation such as cytokines, growth factors, eicosanoids, ROS, and NOS form complex networks not only for maintaining or reducing inflammation but also promoting cell growth, angiogenesis, and inhibiting apoptosis. These events eventually merge into and result in both genetic and epigenetic changes of the cellular genome, leading to inflammation-associated cancer development. In particular, AID plays a crucial role in inducing not only mutations, but also chromosomal aberrations during inflammation. Moreover, signals from macrophages with resulting mislocalization of DNMTs appear to be involved in the induction of epigenetic alterations.

Interestingly, epigenetic inactivation of *MLH1* leads to accumulation of genetic alterations.[170] At the same time, recent studies have shown that AID induces DNA demethylation through its deaminating activity on methylated cytosines.[131] Thus, genetic and epigenetic events are mutually related and work in concert in the development of inflammation-associated cancers.

References

1. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860–867.
2. Walczak H. TNF and ubiquitin at the crossroads of gene activation, cell death, inflammation, and cancer. Immunol Rev 2011; 244:9–28.
3. Yeh JM, Goldie SJ, Kuntz KM, et al. Effects of *Helicobacter pylori* infection and smoking on gastric cancer incidence in China: a population-level analysis of trends and projections. Cancer Causes Control 2009;20:2021–2029.
4. Vennervald BJ, Polman K. Helminths and malignancy. Parasite Immunol 2009;31:686–696.
5. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.
6. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007;121:2373–2380.
7. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. Nature 2008;454:436–444.
8. Stephens PJ, McBride DJ, Lin ML, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009;462:1005–1010.
9. Pleasance ED, Stephens PJ, O'Meara S, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 2010;463:184–190.
10. Sjoblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314:268–274.
11. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010;463:191–196.
12. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer 2008;8:497–511.

13. Ushijima T, Hattori N. molecular pathways: involvement of *Helicobacter pylori*-triggered inflammation in the formation of an epigenetic field defect, and its usefulness as cancer risk and exposure markers. Clin Cancer Res 2012;18:923–929.
14. Ushijima T, Asada K. Aberrant DNA methylation in contrast with mutations. Cancer Sci 2010;101:300–305.
15. Maekita T, Nakazawa K, Mihara M, et al. High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. Clin Cancer Res 2006;12:989–995.
16. Niwa T, Tsukamoto T, Toyoda T, et al. Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. Cancer Res 2010;70: 1430–1440.
17. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
18. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009;27:1485–1491.
19. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273–1275.
20. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.
21. Chiba T, Marusawa H, Seno H, et al. Mechanism for gastric cancer development by *Helicobacter pylori* infection. J Gastroenterol Hepatol 2008;23:1175–1181.
22. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118:3030–3044.
23. Bernstein CN, Blanchard JF, Kliewer E, et al. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer 2001;91:854–862.
24. Askling J, Linet M, Gridley G, et al. Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. Gastroenterology 2002;123: 1428–1435.
25. Elfstrom P, Granath F, Ye W, et al. Low risk of gastrointestinal cancer among patients with celiac disease, inflammation, or latent celiac disease. Clin Gastroenterol Hepatol 2012;10:30–36.
26. Patel T. Cholangiocarcinoma. Nat Clin Pract Gastroenterol Hepatol 2006;3:33–42.
27. Imam MH, Silveira MG, Sinakos E, et al. Long-term outcomes of patients with primary biliary cirrhosis and hepatocellular carcinoma. Clin Gastroenterol Hepatol 2012;10:182–185.
28. Shaheen NJ, Richter JE. Barrett's oesophagus. Lancet 2009; 373:850–861.
29. DiMagno EP, Reber HA, Tempero MA. AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. Gastroenterology 1999;117:1464–1484.
30. Whitcomb DC, Applebaum S, Martin SP. Hereditary pancreatitis and pancreatic carcinoma. Ann N Y Acad Sci 1999;880:201–209.
31. Wotherspoon AC, Doglioni C, Diss TC, et al. Regression of pro-mary low-grade B cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 1993;342:575–577.
32. Kodama Y, Kawabata K, Yoshida S, et al. Malt lymphoma simulating an extramedullary plasmacytoma of the stomach. Am J Med 1999;107:530–532.
33. Hartridge-Lambert SK, Stein EM, Markowitz AJ, et al. Hepatitis C and non-hodgkin lymphoma: the clinical perspective. Hepatology 2012;55:634–641.
34. Smedby KE, Akerman M, Hildebrand H, et al. Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy-type T cell lymphoma. Gut 2005;54:54–59.
35. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398–402.
36. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-kappaB as the matchmaker. Nat Immunol 2011;12:715–723.
37. Kuraishy A, Karin M, Grivennikov SI. Tumor promotion via injury- and death-induced inflammation. Immunity 2011;35:467–477.
38. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–285.
39. Wang D, DuBois RN. Eicosanoids and cancer. Nat Rev Cancer 2010;10:181–193.
40. Ziech D, Franco R, Pappa A, et al. Reactive oxygen species (ROS)—induced genetic and epigenetic alterations in human carcinogenesis. Mutat Res 2011;711:167–173.
41. Sekikawa A, Fukui H, Fujii S, et al. REG Ialpha protein mediates an anti-apoptotic effect of STAT3 signaling in gastric cancer cells. Carcinogenesis 2008;29:76–83.
42. Kanda K, Komekado H, Sawabu T, et al. Nardilysin and ADAM proteases promote gastric cancer cell growth by activating intrinsic cytokine signalling via enhanced ectodomain shedding of TNF-alpha. EMBO Mol Med 2012;4:396–411.
43. Schievella AR, Chen JH, Graham JR, et al. MADD, a novel death domain protein that interacts with the type I tumor necrosis factor receptor and activates mitogen-activated protein kinase. J Biol Chem 1997;272:12069–12075.
44. Kamimura D, Ishihara K, Hirano T. IL6-signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 2003;149:1–38.
45. Chen F. JNK-induced apoptosis, compensatory growth, and cancer stem cells. Cancer Res 2012;72:379–386.
46. Liu J, Yan J, Jiang S, et al. Site-specific ubiquitination is required for releaving the transcription factor Miz1-mediated suppresion on TNFα-induced JNK activation and inflammation. Proc Natl Acad Sci U S A 2012;109:191–196.
47. Inokuchi S, Aoyama T, Miura K, et al. Disruption of TAK1 in *hepatocytes causes hepatic injury, inflammation, fibrosis and* carcinogenesis. Proc Natl Acad Sci U S A 2010;107:844–849.
48. Chang Q, Zhang Y, Beezhold KJ, et al. Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. J Hepatol 2009;50:323–333.
49. Higashi H, Tsutsumi R, Muto S, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–686.
50. Snider JL, Allison C, Bellaire BH, et al. The beta1 integrin activates JNK independent of CagA, and JNK actvation is required for *Helicobacter pylori* CagA+-induced motility of gastric cancer cells. J Biol Chem 2008;283:13952–13963.
51. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57–70.
52. Joerger AC, Fersht AR. Structure-function-rescue: the diverse nature of common p53 cancer mutants. Oncogene 2007;26: 2226–2242.
53. Brentnall TA, Haggitt RC, Rabinovitch PS, et al. Risk and natural history of colonic neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1996;110: 331–338.
54. Hussain SP, Amstad P, Raja K, et al. Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. Cancer Res 2000; 60:3333–3337.
55. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett oesophagus. Nat Genet 1999;22:106–109.
56. Kou T, Marusawa H, Kinoshita K, et al. Expression of activation-induced cytidine deaminase in human hepatocytes during hepatocarcinogenesis. Int J Cancer 2007;120:469–476.
57. Leedham SJ, Graham TA, Oukrif D, et al. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. Gastroenterology 2009;136:542–550.

58. Machida K, Cheng KT, Sung VM, et al. Hepatitis C virus infection activates the immunologic (type II) isoform of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes. J Virol 2004;78:8835–8843.
59. Hussain SP, He P, Subleski J, et al. Nitric oxide is a key component in inflammation-accelerated tumorigenesis. Cancer Res 2008;68:7130–7136.
60. Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 1994;63:915–948.
61. Federico A, Morgillo F, Tuccillo C, et al. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 2007; 121:2381–2386.
62. Gasche C, Chang CL, Rhees J, et al. Oxidative stress increases frameshift mutations in human colorectal cancer cells. Cancer Res 2001;61:7444–7448.
63. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science 2008;319: 1352–1355.
64. Vafa O, Wade M, Kern S, et al. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. Mol Cell 2002;9:1031–1044.
65. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 2004; 567:1–61.
66. Takahashi T, Nau MM, Chiba I, et al. p53: a frequent target for genetic abnormalities in lung cancer. Science 1989;246:491–494.
67. Hsu IC, Metcalf RA, Sun T, et al. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 1991;350: 427–428.
68. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153–158.
69. Conticello SG. The AID/APOBEC family of nucleic acid mutators. Genome Biol 2008;9:229.
70. Liu M, Duke JL, Richter DJ, et al. Two levels of protection for the B cell genome during somatic hypermutation. Nature 2008;451: 841–845.
71. Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. Annu Rev Immunol 2002;20:165–196.
72. Greeve J, Philipsen A, Krause K, et al. Expression of activation-induced cytidine deaminase in human B-cell non-Hodgkin lymphomas. Blood 2003;101:3574–3580.
73. Pasqualucci L, Guglielmino R, Houldsworth J, et al. Expression of the AID protein in normal and neoplastic B cells. Blood 2004; 104:3318–3325.
74. Robbiani DF, Bothmer A, Callen E, et al. AID is required for the chromosomal breaks in *c-myc* that lead to *c-myc/IgH* translocations. Cell 2008;135:1028–1038.
75. Klemm L, Duy C, Iacobucci I, et al. The B cell mutator AID promotes B lymphoid blast crisis and drug resistance in chronic myeloid leukemia. Cancer Cell 2009;16:232–245.
76. Nagaoka H, Tran TH, Kobayashi M, et al. Preventing AID, a physiological mutator, from deleterious activation: regulation of the genomic instability that is associated with antibody diversity. Int Immunol 2010;22:227–235.
77. Endo Y, Marusawa H, Kinoshita K, et al. Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. Oncogene 2007;26:5587–5595.
78. Marusawa H, Hijikata M, Chiba T, et al. Hepatitis C virus core protein inhibits Fas- and tumor necrosis factor alpha-mediated apoptosis via NF-kappaB activation. J Virol 1999;73:4713–4720.
79. Matsumoto Y, Marusawa H, Kinoshita K, et al. *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med 2007;13: 470–476.
80. Endo Y, Marusawa H, Kou T, et al. Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenterology 2008; 135:889–898.
81. Komori J, Marusawa H, Machimoto T, et al. Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. Hepatology 2008;47:888–896.
82. Morita S, Matsumoto Y, Okuyama S, et al. Bile acid-induced expression of activation-induced cytidine deaminase during the development of Barrett's oesophageal adenocarcinoma. Carcinogenesis 2011;32:1706–1712.
83. Okazaki IM, Hiai H, Kakazu N, et al. Constitutive expression of AID leads to tumorigenesis. J Exp Med 2003;197:1173–1181.
84. Morisawa T, Marusawa H, Ueda Y, et al. Organ-specific profiles of genetic changes in cancers caused by activation-induced cytidine deaminase expression. Int J Cancer 2008;123:2735–2740.
85. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988;53:549–554.
86. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 2002;31:339–346.
87. Matsumoto Y, Marusawa H, Kinoshita K, et al. Up-regulation of activation-induced cytidine deaminase causes genetic aberrations at the *CDKN2b-CDKN2a* in gastric cancer. Gastroenterology 2010;139:1984–1994.
88. Feldhahn N, Henke N, Melchior K, et al. Activation-induced cytidine deaminase acts as a mutator in BCR-ABL1-transformed acute lymphoblastic leukemia cells. J Exp Med 2007;204:1157–1166.
89. Takai A, Marusawa H, Minaki Y, et al. Targeting activation-induced cytidine deaminase prevents colon cancer development despite persistent colonic inflammation. Oncogene 2012;31: 1733–1742.
90. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128: 683–692.
91. Rauch TA, Wu X, Zhong X, et al. A human B cell methylome at 100-base pair resolution. Proc Natl Acad Sci U S A 2009;106: 671–678.
92. Lister R, Pelizzola M, Dowen RH, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 2009;462:315–322.
93. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128:707–719.
94. Lin JC, Jeong S, Liang G, et al. Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. Cancer Cell 2007;12:432–444.
95. Yamashita S, Hosoya K, Gyobu K, et al. Development of a novel output value for quantitative assessment in methylated DNA immunoprecipitation-CpG island microarray analysis. DNA Res 2009;16:275–286.
96. Kondo Y, Shen L, Cheng AS, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet 2008;40:741–750.
97. Margueron R, Reinberg D. Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet 2010;11:285–296.
98. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer 2004;4:143–153.
99. Yoshida T, Yamashita S, Takamura-Enya T, et al. Alu and Satα hypomethylation in *Helicobacter pylori*-infected gastric mucosae. Int J Cancer 2011;128:33–39.
100. Rauch TA, Zhong X, Wu X, et al. High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. Proc Natl Acad Sci U S A 2008;105:252–257.
101. Takeshima H, Ushijima T. Methylation destiny: Moira takes account of histones and RNA polymerase II. Epigenetics 2010;5: 89–95.

102. Takeshima H, Yamashita S, Shimazu T, et al. The presence of RNA polymerase II, active or stalled, predicts epigenetic fate of promoter CpG islands. Genome Res 2009;19:1974–1982.
103. Chen RZ, Pettersson U, Beard C, et al. DNA hypomethylation leads to elevated mutation rates. Nature 1998;395:89–93.
104. Enroth S, Rada-Iglesisas A, Andersson R, et al. Cancer associated epigenetic transitions identified by genome-wide histone methylation binding profiles in human colorectal cancer samples and paired normal mucosa. BMC Cancer 2011;11:450.
105. Issa JP, Ottaviano YL, Celano P, et al. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 1994;7:536–540.
106. Hsieh CJ, Klump B, Holzmann K, et al. Hypermethylation of the p16INK4a promoter in colectomy specimens of patients with long-standing and extensive ulcerative colitis. Cancer Res 1998; 58:3942–3945.
107. Issa JP, Ahuja N, Toyota M, et al. Accelerated age-related CpG island methylation in ulcerative colitis. Cancer Res 2001;61: 3573–3577.
108. Kondo Y, Kanai Y, Sakamoto M, et al. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis—a comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. Hepatology 2000;32:970–979.
109. Nakajima T, Maekita T, Oda I, et al. Higher methylation levels in gastric mucosae significantly correlate with higher risk of gastric cancers. Cancer Epidemiol Biomarkers Prev 2006;15:2317–2321.
110. Shen L, Kondo Y, Rosner GL, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. J Natl Cancer Inst 2005;97:1330–1338.
111. Yan PS, Venkataramu C, Ibrahim A, et al. Mapping geographic zones of cancer risk with epigenetic biomarkers in normal breast tissue. *Clin Cancer Res* 2006;12:6626–6636.
112. Arai E, Kanai Y, Ushijima S, et al. Regional DNA hypermethylation and DNA methyltransferase (DNMT) 1 protein overexpression in both renal tumors and corresponding nontumorous renal tissues. Int J Cancer 2006;119:288–296.
113. Ushijima T. Epigenetic field for cancerization. J Biochem Mol Biol 2007;40:142–150.
114. Shin CM, Kim N, Park JH, et al. Prediction of the risk for gastric ancer using candidate methylation markers in the non-neoplastic gastric mucosae. J Pathol 2012;226:654–665.
115. Hur K, Niwa T, Toyoda T, et al. Insufficient role of cell proliferation in aberrant DNA methylation induction and involvement of specific types of inflammation. Carcinogenesis 2011;32:35–41.
116. Yoo EJ, Park SY, Cho NY, et al. Influence of IL1B polymorphism on CpG island hypermethylation in *Helicobacter pylori*-infected gastric cancer. Virchows Arch 2010;456:647–652.
117. Rosenberg DW, Giardina C, Tanaka T. Mouse models for the study of colon carcinogenesis. Carcinogenesis 2009;30:183–196.
118. Katsurano M, Niwa T, Yasui Y, et al. Early-stage formation of an epigenetic field defect in a mouse colitis model, and non-essential roles of T- and B-cells in DNA methylation induction. Oncogene 2012;31:342–351.
119. Hmadcha A, Bedoya FJ, Sobrino F, et al. Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. J Exp Med 1999;190:1595–1604.
120. Hodge DR, Xiao W, Clausen PA, et al. Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. J Mol Biol 2001;276:39508–39511.
121. Braconi C, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. Hepatology 2010;51:881–890.
122. Xia D, Wang D, Kim SH, et al. Prostaglandin E(2) promotes intestinal tumor growth via DNA methylation. Nat Med 2012;18: 224–226.
123. Nakajima T, Yamashita S, Maekita T, et al. The presence of a methylation fingerprint of *Helicobacter pylori* infection in human gastric mucosae. Int J Cancer 2009;124:905–910.
124. O'Hagan HM, Wang W, Sen S, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. Cancer Cell 2011;20: 606–619.
125. Stirzaker C, Song JZ, Davidson B, et al. Transcriptional gene silencing promotes DNA hypermethylation through a sequential change in chromatin modifications in cancer cells. Cancer Res 2004;64:3871–3877.
126. Ushijima T, Watanabe N, Shimizu K, et al. Decreased fidelity in replicating CpG methylation patterns in cancer cells. Cancer Res 2005;65:11–17.
127. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics 2009;1:239–259.
128. Bae JM, Shin SH, Kwon HJ, et al. ALU and LINE-1 hypomethylations in multistep gastric carcinogenesis and their prognostic implications. Int J Cancer 2012;131:1323–1331.
129. Cortellino S, Xu J, Sannai M, et al. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell 2011;146:67–79.
130. Guo JU, Su Y, Zhong C, et al. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell 2011;145:423–434.
131. Fritz EL, Papavasiliou N. Cytidine deaminases: AIDing DNA demethylation? Genes Dev 2010;24:2107–2114.
132. Kato L, Begum NA, Burroughs AM, et al. Nonimmunoglobulin target loci of activation-induced cytidine deaminase (AID) share unique features with immunoglobulin genes. Proc Natl Acad Sci U S A 2012;109:2479–2484.
133. *Di Leva G, Croce CM. Roles of small RNAs in tumor formation.* Trends Mol Med 2010;16:257–267.
134. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704–714.
135. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–838.
136. Sonkoly E, Pivarcsi A. MicroRNAs in inflammation and response to injuries induced by environmental pollution. Mutat Res 2011; 717:46–53.
137. O'Connell RM, Rao DS, Baltimore D. MicroRNA regulation of inflammatory responses. Annu Rev Immunol 2012;30:295–312.
138. Zhao JL, Rao DS, Boldin MP, et al. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci U S A 2011;108:9184–9189.
139. Padgett KA, Lan RY, Leung PC, et al. Primary biliary cirrhosis is associated with altered hepatic microRNA expression. J Autoimmun 2009;32:246–253.
140. Wu F, Zikusoka M, Trindade A, et al. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. Gastroenterology 2008; 135:1624–1635.
141. Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis 2010;31:37–49.
142. Kong D, Piao YS, Yamashita S, et al. Inflammation-induced repression of tumor suppressor miR-7 in gastric tumor cells. Oncogene 2011; doi;10.1038/onc.2011.558.
143. Tili E, Michaille JJ, Wernicke D, et al. Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. Proc Natl Acad Sci U S A 2011;108:4908–4913.
144. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–647.

145. Akao Y, Nakagawa Y, Naoe T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biol Pharm Bull 2006;29:903–906.
146. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999–3004.
147. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell 2009;139:693–706.
148. Tili E, Croce CM, Michaille JJ. MiR-155: on the crosstalk between inflammation and cancer. Int Rev Immunol 2009;28:264–284.
149. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–2261.
150. O'Connell RM, Taganov KD, Boldin MP, et al. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A 2007;104:1604–1609.
151. Xiao B, Liu Z, Li BS, et al. Induction of microRNA-155 during *Helicobacter pylori* infection and its negative regulatory role in the inflammatory response. J Infect Dis 2009;200:916–925.
152. Valeri N, Gasparini P, Fabbri M, et al. Modulation of mismatch repair and genomic stability by miR-155. Proc Natl Acad Sci U S A 2010;107:6982–6987.
153. Teng G, Hakimpour P, Landgraf P, et al. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. Immunity 2008;28:621–629.
154. Dorsett Y, McBride KM, Jankovic M, et al. MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. Immunity 2008;28:630–638.
155. Pekow JR, Kwon JH. MicroRNAs in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:187–193.
156. Iliopoulos D, Jaeger SA, Hirsch HA, et al. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell 2010; 39:493–506.
157. Olaru AV, Selaru FM, Mori Y, et al. Dynamic changes in the expression of MicroRNA-31 during inflammatory bowel disease-associated neoplastic transformation. Inflamm Bowel Dis 2011; 17:221–231.
158. Rothwell PM, Fowkes FG, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011;377:31–41.
159. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol Sci 2009;30:85–94.
160. Kanai M, Imaizumi A, Otsuka Y, et al. Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. Cancer Chemother Pharmacol 2012;69:65–70.
161. He ZY, Shi CB, Wen H, et al. Upregulation of p53 expression in patients with colorectal cancer by administration of curcumin. Cancer Invest 2011;29:208–213.
162. Delmas D, Lancon A, Colin D, et al. Resveratrol as a chemopreventive agent: a promising molecule for fighting cancer. Current Drug Targets 2006;7:423–442.
163. Tili E, Michaille JJ, Adair B, et al. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. Carcinogenesis 2010;31:1561–1566.
164. Aggarwal BB, Van Kuiken ME, Iyer LH, et al. Molecular targets of nutraceuticals derived from dietary spices: potential role in suppression of inflammation and tumorigenesis. Exp Biol Med 2009;234:825–849.
165. Fukase K, Kato M, Kikuchi S, et al. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 2008;372:392–397.
166. Ikeda K, Marusawa H, Osaki Y, et al. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. Ann Intern Med 2007;146:649–656.
167. Sato Y, Takahashi S, Kinouchi Y, et al. IL-10 deficiency leads to somatic mutations in a model of IBD. Carcinogenesis 2006;27: 1068–1073.
168. Ando T, Yoshida T, Enomoto S, et al. DNA methylation of microRNA genes in gastric mucosae of gastric cancer patients: its possible involvement in the formation of epigenetic field defect. Int J Cancer 2009;124:2367–2374.
169. Nanjo S, Asada K, Yamashita S, et al. Identification of gastric cancer risk markers that are informative in individuals with past *H. pylori* infection. Gastric Cancer 2012. [Epub ahead of print].
170. Toyota M, Ahuja N, Ohe-Toyota M, et al. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A 1999; 96:8681–8686.

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Conflicts of interest

The authors disclose no conflicts.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Decrease in alpha-fetoprotein levels predicts reduced incidence of hepatocellular carcinoma in patients with hepatitis C virus infection receiving interferon therapy: a single center study

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Abstract
Background Increasing evidence suggests the efficacy of interferon therapy for hepatitis C in reducing the risk of hepatocellular carcinoma (HCC). The aim of this study was to identify predictive markers for the risk of HCC incidence in chronic hepatitis C patients receiving interferon therapy.
Methods A total of 382 patients were treated with standard interferon or pegylated interferon in combination with ribavirin for chronic hepatitis C in a single center and evaluated for variables predictive of HCC incidence.
Results Incidence rates of HCC after interferon therapy were 6.6% at 5 years and 13.4% at 8 years. Non-sustained virological response (non-SVR) to antiviral therapy was an independent predictor for incidence of HCC in the total study population. Among 197 non-SVR patients, independent predictive factors were an average alpha-fetoprotein (AFP) integration value ≥10 ng/mL and male gender. Even in patients whose AFP levels before interferon therapy were ≥10 ng/mL, reduction of average AFP integration value to <10 ng/mL by treatment was strongly associated with a reduced incidence of HCC. This was significant compared to patients with average AFP integration values of ≥10 ng/mL ($P = 0.009$).
Conclusions Achieving sustained virological response (SVR) by interferon therapy reduces the incidence of HCC in hepatitis C patients treated with interferon. Among non-SVR patients, a decrease in the AFP integration value by interferon therapy closely correlates with reduced risk of HCC incidence after treatment.

Keywords Alpha-fetoprotein · Hepatocellular carcinoma · Hepatitis C · Interferon

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Introduction

Hepatitis C virus (HCV) infection is a predominant cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries, including Japan, the United States, and countries of Western Europe [1–5]. The annual incidence of HCC in patients with HCV-related cirrhosis ranged from 1 to 8% [6–9]. Even in the absence of liver cirrhosis, patients with chronic hepatitis caused by HCV infection are at a high risk of developing HCC. Indeed, a large-scale Japanese cohort study showed that the annual incidence of HCC is 0.5% among patients with stage F0 or F1 fibrosis and 2.0, 5.3, and 7.9% among those with F2, F3, and F4 fibrosis, respectively [9]. Periodic surveillance is recommended to detect HCC as early as possible in patients with HCV-related chronic liver disease; however, this may not be cost-effective. For patients with chronic hepatitis C, more effective detection and prevention of HCC is being sought by two important routes: (1) the attempt to discover noninvasive predictive markers and (2) development of treatment strategies to reduce the risk of HCC. There have been several attempts to discover non-invasive markers capable of predicting the risk of HCC incidence in patients with chronic hepatitis C [6, 10]. For example, a cohort

derived from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial identified older age, African American race, lower platelet count, higher alkaline phosphatase, and esophageal varices as risk factors for HCC [11].

There have also been a number of studies to evaluate the effect of anti-viral treatment of chronic hepatitis C on the incidence of HCC [12–19]. The results were summarized in a meta-analysis, which concluded that the effect of interferon on risk of HCC is mainly apparent in patients achieving a sustained virological response (SVR) to interferon therapy [13]. In addition, a number of studies have suggested the incidence of HCC is reduced in treated patients compared to historical controls [12, 15, 16, 19]. However, the recent HALT-C randomized control trial revealed that long-term pegylated interferon therapy does not reduce the incidence of HCC among patients with advanced hepatitis C who do not achieve SVRs. Reduction in the risk of HCC by maintenance therapy was shown only in patients with cirrhosis [14, 17]. These controversial results suggest that interferon therapy reduces the risk of HCC only in a group of patients with HCV-related chronic liver disease. Thus, it is important to evaluate the risk of HCC development in hepatitis C patients receiving interferon therapy and it will be clinically useful to discover markers distinguishing high- and low-risk groups.

Serum alpha-fetoprotein (AFP) has been widely used as a diagnostic marker of HCC [20–22]. However, elevation of serum AFP levels is often found in non-neoplastic liver diseases without evidence of HCC, including acute liver injury and chronic viral hepatitis [23–27], especially among patients with advanced chronic hepatitis C [28]. An increase of AFP after liver damage is interpreted as a sign of dedifferentiated hepatic regeneration [27]. There have been some reports that AFP is a significant predictor of HCC in patients with chronic hepatitis C [4, 5, 29]. In addition, it has recently been shown that AFP levels decrease in response to interferon administration in patients with chronic hepatitis C [30, 31], and that long-term interferon therapy for aged patients with chronic HCV infection is effective in decreasing serum AFP levels and preventing hepatocarcinogenesis [32, 33]. However, little is known about the relationship between changes in serum AFP level over time during interferon therapy and the development of HCC.

The aim of this large single center study was to identify predictive markers for the risk of HCC development in patients receiving interferon therapy for chronic hepatitis C. For this purpose, patients treated with standard or pegylated interferon, in combination with ribavirin, for chronic hepatitis C were enrolled and subjected to scheduled periodic surveillance for HCC and a number of potential predictive markers, including AFP and alanine aminotransferase (ALT) integration values, at a single center.

Materials and methods

Patients

Between January 2002 and April 2010, 528 patients with chronic hepatitis C received combination therapy with standard interferon and ribavirin ($n = 84$) or pegylated interferon and ribavirin ($n = 444$) at Osaka Red Cross Hospital. Eligibility criteria for treatment were positivity for serum HCV RNA and histological evidence of chronic hepatitis C ($n = 427/444$; 80.9%), or positivity for serum HCV RNA, liver enzyme levels greater than the normal upper limit, and an ultrasound image demonstrating chronic liver damage ($n = 101/444$; 19.1%). Exclusion criteria for treatment were as follows: neutrophil count <750 cells/μL, platelet count <50,000 cells/μL, hemoglobin level ≤9.0 g/dL, and renal insufficiency (serum creatinine levels >2 mg/dL).

Of 528 patients who received interferon therapy for chronic hepatitis C, 146 were excluded from this study for the following reasons: follow-up <24 weeks after the termination of the interferon therapy ($n = 122$), previously treated for HCC ($n = 22$), or occurrence of HCC during or within 24 weeks after treatment ($n = 2$). Therefore, 382 patients were enrolled for the study and were retrospectively analyzed.

To detect early-stage HCC, ultrasonography, dynamic contrast enhanced computed tomography (CT), dynamic contrast enhanced magnetic resonance imaging (MRI), and/or measurement of tumor markers (including AFP) were performed for all patients at least every 6 months. HCC was diagnosed radiologically as liver tumors displaying arterial hypervascularity and venous or delayed phase washout by dynamic contrast enhanced CT or MRI.

The study protocol was approved by the Ethics Committee at Osaka Red Cross Hospital and performed in compliance with the Helsinki Declaration.

Treatment protocol and definition of responses to treatment

The basic treatment protocol for patients with chronic hepatitis C consisted of 6 mega units of interferon-α-2b 3 times a week or 1.5 μg/kg of pegylated interferon α-2b once a week, combined with ribavirin at an oral dosage of 600–1000 mg/day. Duration of the treatment was 48–72 weeks for those with HCV genotype 1 and serum HCV RNA titer of >5 log IU/mL, and 24 weeks for all other patients.

Patients who were negative for serum HCV RNA for >6 months after completion of interferon therapy were defined as showing an SVR. Patients whose serum ALT levels decreased to the normal range and remained normal for >6 months after the termination of interferon therapy were defined as showing a sustained biochemical response (SBR).

Patients who did not achieve SVR received ursodeoxycholic acid and/or glycyrrhizin containing preparation (Stronger Neo-Minophagen C), when serum ALT levels were higher than the upper limit of normal.

Virological assays

HCV genotype was determined by polymerase chain reaction (PCR) amplification of the core region of the HCV genome using genotype-specific PCR primers [34]. Serum HCV RNA load was evaluated once a month during and 24 weeks after treatment using a PCR assay (Cobas Amplicor HCV Monitor, Roche Molecular Systems, Pleasanton, CA, USA).

Measurement of AFP and calculation of average integration value

AFP was measured in serum samples obtained from each patient at intervals of 1–3 months. The median number of examinations was 15 (range 1–70) in each patient. Serum AFP levels were determined by enzyme-linked immunosorbent assay, which was performed using a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Integration values of AFP and ALT were calculated as described in previous reports [35]. For example, the integration value of AFP was calculated as follows, $(y_0 + y_1) \times x_1/2 + (y_1 + y_2) \times x_2/2 + (y_2 + y_3) \times x_3/2 + (y_3 + y_4) \times x_4/2 + (y_4 + y_5) \times x_5/2 + (y_5 + y_6) \times x_6/2$, i.e., the area of each trapezoid representing an AFP value was measured the sum of the resulting values used to calculate the integration value (Fig. 1). The average integration value was obtained by dividing the integration value by the observation period from initiation of the treatment.

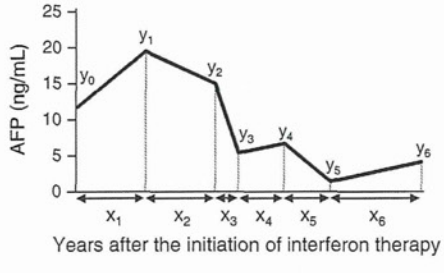

Fig. 1 Example plot of data used for calculation of average integration value of alpha-fetoprotein (AFP)

Statistical analysis

The Kaplan–Meier method was used to estimate the rates of development of HCC in patients after interferon therapy. Log-rank tests were used to evaluate the effects of predictive factors on incidence of HCC. Significance was defined as $P < 0.05$. Multivariate Cox regression analysis using the stepwise method was used to evaluate the association between HCC incidence and patient characteristics, and to estimate hazard ratio (HR) with a 95% confidence interval (CI). A P value of 0.1 was used for variable selection and was regarded as statistically significant. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Characteristics of patients and incidence of HCC

This study included 382 patients treated for chronic hepatitis C with standard interferon or pegylated interferon in combination with ribavirin. Baseline clinical and virological characteristics of patients included in the study are summarized in Table 1. The median age of the patients at the outset of therapy was 59.0 years (range 18–81 years) and the median follow-up period was 4.1 years (range 0.1–8.4 years). The majority of patients were infected with HCV genotype 1b ($n = 229$; 60%), and median serum HCV RNA load was 6.1 log IU/mL (range 2.3–7.3 log IU/mL). Baseline (before interferon therapy) median serum AFP level was 6.9 ng/mL (range 1.6–478.3 ng/mL).

During follow-up, 23 patients (4.9%) developed HCC. The cumulative incidences of HCC, which was estimated using the Kaplan–Meier method, were 3.1, 6.6, and 13.4% at 3, 5, and 8 years, respectively (Fig. 2).

Predictive factors for incidence of HCC in all patients

Predictive factors for incidence of HCC in all 382 patients were analyzed using log-rank tests (Table 2). Univariate analysis showed that age ≥70 years ($P = 0.040$), non-SVR ($P < 0.0001$), non-SBR ($P = 0.027$), average ALT integration value ≥40 IU/L ($P = 0.001$), baseline AFP ≥10 ng/mL ($P = 0.005$), average AFP integration value ≥10 ng/mL ($P < 0.0001$), and baseline platelet count <150,000 platelets/μL ($P = 0.001$) were all significantly associated with the incidence of HCC. After multivariate analysis, the only variable remaining in the model was non-SVR (HR 8.413, 95% CI 1.068–66.300, $P = 0.043$).

Table 1 Characteristics of 382 patients with hepatitis C treated with interferon therapy in this study

Age (years)	59.0 (18–81)
[a]Males/females	192/190
Observation period (years)	4.1 (0.1–8.4)
[a]IFN + RBV/PEG-IFN + RBV	69/313
HCV genotype 1/2/unclassified	229/57/96
HCV RNA (log IU/mL)	6.1 (2.3–7.3)
White blood cell count (/μL)	4950 (2050–9970)
Hemoglobin (g/dL)	14.0 (10.3–18.8)
Platelet (10^4/μL)	15.0 (5.3–36.4)
AST (IU/L)	56 (17–244)
ALT (IU/L)	67 (16–416)
Bilirubin (mg/dL)	0.8 (0.3–2.4)
AFP (ng/mL)	6.9 (1.6–478.3)

Qualitative variables ([a]) are shown in number, and quantitative variables expressed as median (range)

IFN interferon, *RBV* ribavirin, *PEG-IFN* pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein

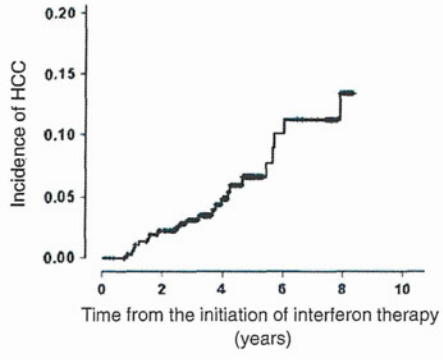

Fig. 2 Incidence of hepatocellular carcinoma (HCC) in 382 patients with hepatitis C who received interferon therapy, estimated using the Kaplan–Meier method

Further, although patients with average AFP integration values ≥10 ng/mL also appeared to have an increased risk of HCC, the difference did not reach statistical significance in the multivariate analysis ($P = 0.050$) (Table 3).

Predictive factors for incidence of HCC in non-SVR patients

Because non-SVR was the only predictive factor across the entire study cohort, to clarify predictive factors for incidence of HCC within this group, the same variables were further analyzed in non-SVR patients alone. By univariate analysis, average AFP integration value ≥10 ng/mL ($P = 0.019$) and baseline platelet count <150,000 ($P = 0.0022$) (Table 2) were again identified as significant predictive factors for incidence of HCC. In addition, male gender was significantly associated with incidence of HCC in non-SVR patients ($P = 0.022$). Multivariate analysis, however, indicated that only two variables were independently associated with incidence of HCC in non-SVR patients: average AFP integration value ≥10 ng/mL (HR 4.039, 95% CI 1.570–10.392, $P = 0.004$), and male gender

Table 2 Univariate analysis of predictive factors for incidence of hepatocellular carcinoma in all 382 and 197 non-SVR patients

Factors	All ($n = 382$)			Non-SVR ($n = 197$)		
	No.	Incidence of HCC ($n = 23$)		No.	Incidence of HCC ($n = 22$)	
		No. (%)	P value[a]		No. (%)	P value[a]
Age (years)						
<70	359	19 (5)	0.040	182	18 (10)	0.089
≥70	23	4 (17)		15	4 (27)	
Sex						
Female	190	8 (4)	0.125	111	8 (7)	0.022
Male	192	15 (8)		86	14 (16)	
HCV genotype						
1	229	12 (5)	0.452	137	12 (9)	0.796
Non-1	57	1 (2)		10	1 (10)	
Virological response						
SVR	185	1 (1)	<0.0001			
Non-SVR	197	22 (11)				
Biochemical response						
SBR	282	12 (4)	0.027	102	11 (11)	0.857
Non-SBR	86	11 (13)		81	11 (14)	
ALT before IFN therapy						
<40	79	2 (3)	0.274	39	2 (5)	0.319
≥40	301	21 (7)		158	20 (13)	
ALT integration value						
<40	238	6 (3)	0.001	79	5 (6)	0.153
≥40	142	17 (12)		118	17 (14)	
AFP before IFN therapy						
<10	230	7 (3)	0.005	102	7 (7)	0.124
≥10	116	14 (12)		75	13 (17)	
AFP integration value						
<10	258	8 (3)	<0.0001	115	8 (6)	0.019
≥10	63	12 (19)		53	11 (21)	
Platelet before IFN therapy						
<150,000	187	20 (11)	0.001	121	19 (16)	0.022
≥150,000	194	3 (2)		76	3 (4)	

[a] Log-rank test

SVR sustained virological response, *SBR* sustained biochemical response, *ALT* alanine aminotransferase, *IFN* interferon, *AFP* alpha-fetoprotein

Table 3 Multivariate analysis of the predictive factors for incidence of hepatocellular carcinoma in all 382 patients

Factors	Hazard ratio	95% CI	*P* value
Virological response			
SVR	1		
Non-SVR	8.413	1.068–66.300	0.043
AFP integration value			
<10	1		
≥10	2.580	0.999–6.659	0.050

SVR sustained virological response, *IFN* interferon, *AFP* alpha-fetoprotein

Table 4 Multivariate analysis of predictive factors for incidence of hepatocellular carcinoma in 197 non-SVR patients

Factors	Hazard ratio	95% CI	*P* value
AFP integration value			
<10	1		
≥10	4.039	1.570–10.392	0.004
Sex			
Female	1		
Male	3.636	1.383–9.563	0.009

AFP alpha-fetoprotein

(HR 3.636, 95% CI 1.383–9.563, $P = 0.009$) (Table 4). There was no significant difference in other variables including those identified as predictive factors in the entire study population (i.e., age, non-SBR, ALT integration value, AFP before interferon therapy) (Table 2).

AFP integration value as a predictive factor for HCC

Further analysis focused on the AFP integration value as this was the strongest predictive factor for incidence of HCC in non-SVR patients. Of the 382 patients, both baseline and AFP integration values were available for 321. These were divided into four groups: (1) AFP "low–low," (2) AFP "low–high," (3) AFP "high–low," and (4) AFP "high–high," for baseline AFP-average AFP integration values, respectively, where "high" is ≥10 ng/mL and "low" is <10 ng/mL. As shown in Fig. 3a, of the 321 patients, 211 (65.7%) showed baseline AFP levels <10 ng/mL. Of these 211, 207 (98%), were in the AFP low–low group, and only four in the AFP low–high groups. Baseline characteristics, including age, gender, serum HCV-RNA, aspartate aminotransferase (AST), ALT, bilirubin, white blood cell, hemoglobin, platelet, observation periods, and number of times of AFP measurement, were not different between AFP high–low group and high–high group. However, AFP-low group, which is a combination of the low–high and low–low groups, showed significantly lower AST level ($P < 0.00001$), lower ALT level ($P < 0.00001$), higher platelet count ($P < 0.00001$), shorter observation period ($P = 0.01448$), and fewer number of times of AFP examination ($P = 0.00035$), compared to both AFP high–high and AFP high–low group. Six patients (2.8%) with baseline AFP levels <10 ng/mL developed HCC in the follow-up period and none of these patients were among the four low–high group patients. Even in patients with high baseline AFP levels, incidence of HCC was only 3.9% among the AFP high–low group (2 of 51 patients). In contrast, 20.3% of patients in the AFP high–high group developed HCC during the follow-up period.

The incidence rate of HCC in three patient groups, "AFP-low" (a combination of the "low–high" and "low–low" groups),"high–low," and "high–high", was estimated using the Kaplan–Meier method and compared using log-rank tests (Fig. 3b). The rate of HCC incidence was significantly higher in the AFP high–high group compared to both the AFP high–low group and patients with low baseline AFP levels ($P = 0.009$ and 0.001, respectively). There was no significant difference between patients with low baseline AFP levels and the AFP high–low group. The 7-year incidence rate of HCC was 32.3% in the AFP high–high group, compared to only 6.6% in the AFP high–low group, and 8.1% in all patients with low pre-treatment levels.

Discussion

It is well recognized that the most effective strategy for the prevention of HCC development in patients with chronic hepatitis C is likely to be the complete elimination of the HCV infection accompanied by the resultant normalization of liver function [7, 12, 13, 15, 16, 19]. Indeed, we confirmed here that non-SVR is the most significant predictive factor for incidence of HCC in patients receiving interferon therapy for chronic hepatitis C. However, it should be noted that the risk of HCC, even in non-SVR patients, differs between individuals. In the current study, we identified AFP integration value and male gender as independent risk factors for incidence of HCC in non-SVR patients. The incidence of HCC was significantly reduced in individuals with average AFP integration values < 10 ng/mL after interferon therapy, which suggests that the decrease of AFP by interferon therapy lowers the risk of developing HCC. Indeed, even where patients had high baseline AFP levels, incidence of HCC was reduced when the AFP integration value decreased after interferon therapy. Thus, our current findings identify AFP integration value as a useful predictive marker of HCC development in non-SVR patients.

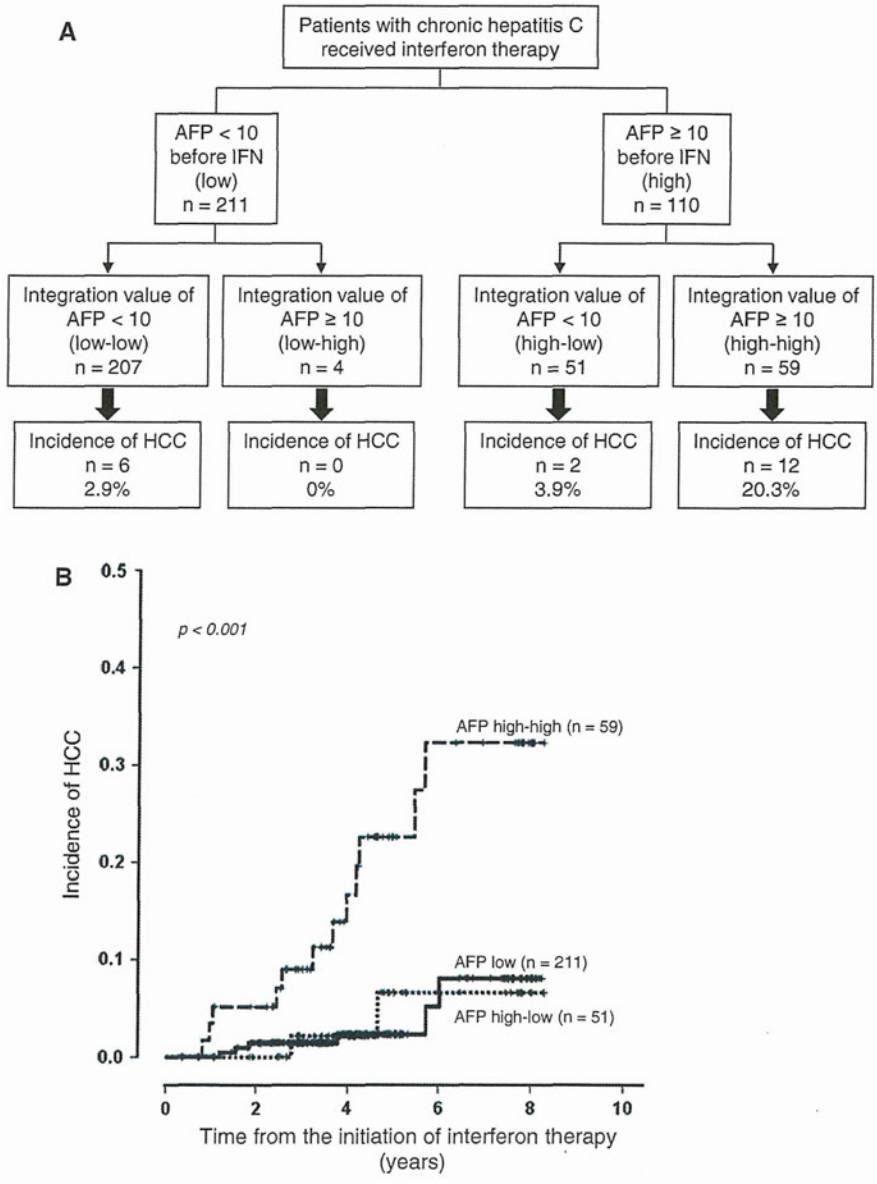

Fig. 3 AFP integration value as a predictive factor for HCC. **a** *Flow diagram* showing the number of patients (*n*) classified by baseline alpha-fetoprotein (AFP) levels before interferon (IFN) therapy and average AFP integration value, and the incidence of hepatocellular carcinoma (HCC) of each group. **b** Kaplan–Meier estimates of the incidence of HCC. *Solid line* AFP-low group (AFP levels before interferon therapy <10 ng/mL); *dotted line* AFP high–low group (baseline AFP levels ≥10 ng/mL, average AFP integration value <10 ng/mL); *dashed line* AFP high–high group (both baseline and average AFP integration values ≥10 ng/mL)

Data from several previous studies suggest that the continuous normalization of alanine aminotransferase (ALT) levels by interferon therapy can reduce the risk of HCC development [36–39]. In addition, one recent study suggested that the ALT integration value is a predictive factor for HCC [35]. In contrast to published data (22), our multivariate analysis did not identify the ALT integration value as a significant predictive factor for HCC incidence, although it was identified as significant by univariate analysis in all 382 patients. Since the previous study did not evaluate AFP levels as a factor for prediction of HCC [35], our results indicate that the AFP integration value is superior to that of ALT as a predictive factor for incidence of HCC. We do not know the reason for this result, but it is speculated that significance of AFP as a marker of hepatic regeneration resulted in the more accurate prediction of hepatocarcinogenesis by integration value of AFP than that of ALT.

As AFP is a diagnostic marker for the existence of HCC, high integration value of AFP in the present study might be a result of HCC development. However, we concluded that the high AFP integration values in patients who developed HCC were not caused by a result of existence of HCC, because of the following two reasons. First, the last AFP values before detection of HCC were not the highest level in the follow-up periods in 19 of 23 patients who developed

HCC, suggesting that the AFP was not produced by the developing HCC in these patients. Second, to exclude the influence of the remaining four patients whose last AFP levels were the highest in the follow-up periods, we analyzed the same statistical analysis by using average AFP integration values excluded the last two examinations of AFP before the detection of HCC. The results of the analysis also showed average integration value of AFP as a significant predictive factor for incidence of HCC.

Male gender was also identified as an independent risk factor for HCC in non-SVR patients in this study. Several reports have shown that men are at a higher risk of developing HCC than women [6, 10, 33, 40, 41]. The male gender also appears to be a risk factor for more severe disease and a greater risk of developing cirrhosis in chronic hepatitis C [42]. Although the association of male gender with the risk of HCC is as yet unexplained, hormonal or genetic factors may lead to increased risk for HCC and cirrhosis in men as previously discussed [10].

In conclusion, a decrease in the AFP integration value predicts reduced incidence of HCC in patients with hepatitis C receiving interferon therapy. Further prospective studies with a larger number of patients are required to validate the significance of these findings.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet. 1989;2:1004–6.
2. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet. 1989;2:1006–8.
3. Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, Houghton M, Choo QL, Kuo G. Hepatitis C-associated hepatocellular carcinoma. Hepatology. 1990;12:589–91.
4. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology. 1993;18:47–53.
5. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328:1797–801.
6. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:S35–50.
7. Ikeda K, Marusawa H, Osaki Y, Nakamura T, Kitajima N, Yamashita Y, Kudo M, Sato T, Chiba T. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. Ann Intern Med. 2007;146:649–56.
8. Liang TJ, Heller T. Pathogenesis of hepatitis C-associated hepatocellular carcinoma. Gastroenterology. 2004;127:S62–71.
9. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. Ann Intern Med. 1999;131:174–81.
10. Heathcote EJ. Prevention of hepatitis C virus-related hepatocellular carcinoma. Gastroenterology. 2004;127:S294–302.
11. Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009; 136:138–48.
12. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. International Interferon-alpha Hepatocellular Carcinoma Study Group. Lancet. 1998;351:1535–9.
13. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001;34:593–602.
14. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 2008;359:2429–41.
15. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, Almasio P, Solinas A, Brouwer JT, Thomas H, Realdi G, Corrocher R, Schalm SW. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma, decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol. 1997;27:201–5.
16. Hayashi K, Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Toyoda H, Shimizu H, Honda T. Incidence of hepatocellular carcinoma in chronic hepatitis C after interferon therapy. Hepatogastroenterology. 2002;49:508–12.
17. Lok AS, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. Gastroenterology. 2011;140:840–9.
18. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346:1051–5.
19. Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Nishioji K, Murakami Y, Kashima K. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. J Hepatol. 1999;30:653–9.
20. Izuno K, Fujiyama S, Yamasaki K, Sato M, Sato T. Early detection of hepatocellular carcinoma associated with cirrhosis by combined assay of des-gamma-carboxy prothrombin and alpha-fetoprotein: a prospective study. Hepatogastroenterology. 1995;42:387–93.

21. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol. 2001;34:570–5.
22. Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. Cancer. 1996;78:977–85.
23. Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology. 1978;74:856–8.
24. Bloomer JR, Waldmann TA, McIntire KR, Klatskin G. Alpha-fetoprotein in noneoplastic hepatic disorders. JAMA. 1975;233:38–41.
25. Ruoslahti E, Seppala M. Normal and increased alpha-fetoprotein in neoplastic and non-neoplastic liver disease. Lancet. 1972;2:278–9.
26. Sakurai T, Marusawa H, Satomura S, Nabeshima M, Uemoto S, Tanaka K, Chiba T. *Lens culinaris* agglutinin-A-reactive alpha-fetoprotein as a marker for liver atrophy in fulminant hepatic failure. Hepatol Res. 2003;26:98–105.
27. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology. 1990;12:1420–32.
28. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43:434–41.
29. Tateyama M, Yatsuhashi H, Taura N, Motoyoshi Y, Nagaoka S, Yanagi K, Abiru S, Yano K, Komori A, Migita K, Nakamura M, Nagahama H, Sasaki Y, Miyakawa Y, Ishibashi H. Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus. J Gastroenterol. 2011;46:92–100.
30. Murashima S, Tanaka M, Haramaki M, Yutani S, Nakashima Y, Harada K, Ide T, Kumashiro R, Sata M. A decrease in AFP level related to administration of interferon in patients with chronic hepatitis C and a high level of AFP. Dig Dis Sci. 2006;51:808–12.
31. Tamura Y, Yamagiwa S, Aoki Y, Kurita S, Suda T, Ohkoshi S, Nomoto M, Aoyagi Y. Serum alpha-fetoprotein levels during and after interferon therapy and the development of hepatocellular carcinoma in patients with chronic hepatitis C. Dig Dis Sci. 2009;54:2530–7.
32. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kumada H. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol. 2007;79:1095–102.
33. Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, Yasui Y, Hosokawa T, Ueda K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Izumi N. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology. 2010;52:518–27.
34. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol. 1997;35:201–7.
35. Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Atsumi H, Takagi M, Nakano S, Arakawa T, Fujimori M. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol. 2009;50:729–35.
36. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kumada H. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol. 2007;79:1485–90.
37. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma, its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology. 1998;27:1394–402.
38. Kurokawa M, Hiramatsu N, Oze T, Mochizuki K, Yakushijin T, Kurashige N, Inoue Y, Igura T, Imanaka K, Yamada A, Oshita M, Hagiwara H, Mita E, Ito T, Inui Y, Hijioka T, Yoshihara H, Inoue A, Imai Y, Kato M, Kiso S, Kanto T, Takehara T, Kasahara A, Hayashi N. Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis. Hepatol Res. 2009;39:432–8.
39. Suzuki K, Ohkoshi S, Yano M, Ichida T, Takimoto M, Naitoh A, Mori S, Hata K, Igarashi K, Hara H, Ohta H, Soga K, Watanabe T, Kamimura T, Aoyagi Y. Sustained biochemical remission after interferon treatment may closely be related to the end of treatment biochemical response and associated with a lower incidence of hepatocarcinogenesis. Liver Int. 2003;23:143–7.
40. Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, Tamaki N, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Enomoto N, Izumi N. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. Hepatol Res. 2010;40:870–7.
41. Takahashi H, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. J Gastroenterol. 2011;46:790–8.
42. Forns X, Ampurdanes S, Sanchez-Tapias JM, Guilera M, Sans M, Sanchez-Fueyo A, Quinto L, Joya P, Bruguera M, Rodes J. Long-term follow-up of chronic hepatitis C in patients diagnosed at a tertiary-care center. J Hepatol. 2001;35:265–71.

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Effect of maintenance therapy with low-dose peginterferon for recurrent hepatitis C after living donor liver transplantation

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SUMMARY. Approximately 30% of patients who have recurrent hepatitis C after liver transplantation achieve sustained virological response (SVR) by taking a combination therapy of pegylated interferon and ribavirin. For the remaining non-SVR patients, an effective management treatment has not yet been established. In this study, efficacy of long-term peginterferon maintenance therapy for non-SVR patients was evaluated. Forty patients who had previously received the combination therapy for hepatitis C after living donor liver transplantation were classified into one of the following three groups: the SVR group (n = 11); the non-SVR-IFN group (n = 17), which received low-dose peginterferon maintenance therapy for non-SVR patients; and the non-SVR-Withdrawal group (n = 12), which discontinued the interferon treatment. We then compared histological changes among these three groups after 2 or more years follow-up. Activity grade of liver histology improved or remained stable in patients in the SVR and non-SVR-IFN groups, but deteriorated in half of the patients in the non-SVR-Withdrawal group. Fibrosis improved or remained stable in 10 of 11 SVR patients and in 13 of 17 non-SVR-IFN patients, but deteriorated in all non-SVR-Withdrawal patients. Mean changes in fibrosis stage between pretreatment and final liver biopsy were −0.18, +0.06 and +2.2 in the SVR, non-SVR-IFN and non-SVR-Withdrawal groups, respectively. Fibrosis stage deteriorated to F3 or F4 significantly more rapidly in the non-SVR-Withdrawal group than in the other two groups. In conclusion, continuing long-term maintenance therapy with peginterferon prevented histological progression of hepatitis C in patients who had undergone living donor liver transplantation.

Keywords: fibrosis, hepatitis C, liver transplantation, maintenance therapy, peginterferon, ribavirin.

INTRODUCTION

Cirrhosis and hepatocellular carcinoma caused by hepatitis C virus (HCV) infection is the leading indication for liver transplantation in Japan, the United States and western Europe. However, liver allograft infection with HCV following liver transplantation is universal, and almost all patients develop recurrent liver injury [1–6]. The progression of recurrent hepatitis C is often accelerated and, without appropriate antiviral therapy, 10–25% of patients develop cirrhosis within 5 years after transplantation, resulting in poorer prognosis for HCV-positive recipients than HCV-negative recipients [7].

To prevent the progression of hepatitis C after liver transplantation, a combined therapy of pegylated interferon plus ribavirin is commonly administered [8,9]. However, the efficacy of this combination therapy is limited: The mean sustained virological response (SVR) rate among patients with recurrent hepatitis C after liver transplantation was only 30% (range, 8–50%) [10]. Effective management of the remaining 70% of the patients who are unable to achieve SVR has not been established [11].

We recently reported the change in liver histology after combination therapy with interferon plus ribavirin in patients who have recurrent hepatitis C after living donor liver transplantations (LDLT). Among patients who did not achieve SVR, activity grade was not improved and fibrosis stage deteriorated. On the other hand, SVR was associated with reduced hepatic inflammation and suppression of liver fibrosis progression [12]. Because the histological progression of non-SVR patients occurred mainly after interferon

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; HCV, hepatitis C virus; LDLT, living donor liver transplantations; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; SVR, sustained virological response.

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therapy was discontinued, we hypothesized that long-term, continuous interferon administration might be effective in slowing the progression of liver damage in these patients. Therefore, after our previous study, we prescribed a low-dose peginterferon maintenance therapy for non-SVR patients. Here, we evaluated the efficacy of this treatment by investigating long-term histological changes in these patients, as well as comparing them to the changes observed in SVR patients and non-SVR patients who did not receive maintenance treatment.

METHODS

Eighty patients who had previously received the combination therapy with interferon and ribavirin (n = 40) or peginterferon and ribavirin (n = 40) for recurrent hepatitis C after LDLT at Kyoto University between January 2001 and April 2007 were retrospectively analysed.

Patients

Between March 1999 and December 2006, 141 patients with HCV-related liver diseases underwent LDLT at Kyoto University. Of these, 100 patients had been followed up for more than 6 months after LDLT in our hospital. Antiviral therapy was given to 80 patients with recurrent hepatitis C between January 2001 and April 2007. The remaining 20 patients did not receive the antiviral therapy because of no histological recurrence of hepatitis C in the follow-up period. To evaluate the histological progression caused by hepatitis C, patients who were diagnosed as having other causes of liver injury, such as biliary complications, chronic rejection, and *de novo* autoimmune hepatitis (AIH), were excluded. Patients who discontinued the treatment within 3 months because of worsening of liver function caused by hepatitis C were also excluded, because the rapid progression of these patients is not comparable to the long-term progression and inclusion of these patients would have led to overestimation of the progression in the patients who discontinued treatment. Patients were also excluded if they did not have a liver biopsy more than 2 years after the initiation of treatment, because this prevented an analysis of long-term histological changes.

Treatment protocol and definition of responses to treatment

After liver transplantation, patients with recurrent HCV liver disease underwent treatment with interferon–α–2b (3 or 6 mega units thrice weekly) plus ribavirin (400–800 mg/day orally) for the first 6 months. This was followed by interferon monotherapy for 6 months [12]. This treatment protocol was employed between January 2001 through April 2004 inclusive. From May 2004 to April 2007, patients underwent combination antiviral therapy comprising peginterferon–α–2b (1.5 μg/kg body weight, weekly) and ribavirin (400–800 mg/day orally) [13]. Patients who became negative for serum HCV RNA within 12 months after initiating the treatment continued to receive the full (initial) dose for 8–22 months to achieve SVR; then, the treatment ended. Patients who were negative for serum HCV RNA for more than 6 months after completion of interferon therapy were defined as achieving SVR.

Patients who did not become negative for serum HCV RNA within 12 months of initiating the combination therapy, as well as patients who experienced a relapse after transient discontinuation of the treatment, continued to receive a low-dose peginterferon maintenance therapy (0.5–0.75 μg/kg of peginterferon-α-2b with or without ribavirin at 200 mg/day). Treatments occurred during the study period, May 2005–December 2009. During this time, the therapy was discontinued in patients with severe adverse events. Additionally, peginterferon treatments were discontinued when neutrophil and platelet counts fell below 500 and 30 000/μL, respectively, and ribavirin was discontinued when haemoglobin levels fell below 8 g/dL.

Histological assessment

Liver biopsies were performed when patients' alanine aminotransferase (ALT) levels were more than twice the upper limit of normal, or at yearly intervals, with informed consent. Biopsy specimens were evaluated by two pathologists (H.H. and A.M.) with extensive experience in the pathology of liver transplantation. Necroinflammatory activity (A0–A3) and fibrosis stage (F0–F4) were assessed using METAVIR scores [14,15]. Grading was defined as A0 (no activity), A1 (mild activity), A2 (moderate activity) or A3 (severe activity); staging was defined as F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis) or F4 (cirrhosis) [14,15].

The following equations were used to analyse the histological changes:

(1) Changes in activity grade = grade at final biopsy − grade at pretreatment biopsy, and
(2) Changes in fibrosis stage = stage at final biopsy − stage at pretreatment biopsy.

Immunosuppression

Tacrolimus and low-dose steroid therapies were administered to induce immunosuppression [12,13,16]. The lower limit of the target for whole blood tacrolimus level was 10–15 ng/mL during the first 2 weeks, 10 ng/mL during weeks 2–8 and 5–8 ng/mL thereafter. Four patients received cyclosporine microemulsions, rather than tacrolimus, to induce immunosuppression (Table 1). Steroid therapy was initiated at a dose of 10 mg/kg before graft reperfusion and then tapered from 1 mg/kg per day on the first day to 0.3 mg/kg per day until the end of the first month, followed by 0.1 mg/kg per day until the end of the third month. After

Table 1 Baseline characteristics of 40 enrolled patients with recurrent hepatitis C after LDLT before interferon therapy

		Non-SVR		
	SVR (n = 11)	IFN (n = 17)	Withdrawal (n = 12)	P
Age (years)	55 (17–68)	57 (39–66)	58 (15–70)	0.724*
Males/Females	7/4	12/5	5/7	0.281†
Time since LDLT (months)	11.5 (4.2–39.1)	10.6 (1.1–51.2)	5.9 (1.8–85.3)	0.316*
HCV genotype 1/non-1	8/3	15/2	12/0	0.141†
HCV RNA (kIU/mL)	1120 (289–5000)	2810 (74–5000)	2320 (498–5000)	0.850*
White cell count (/μL)	4000 (2200–9000)	4600 (1300–6900)	4400 (1700–6900)	0.991*
Neutrophil count (/μL)	2220 (1235–4140)	2040 (793–4816)	2642 (836–4623)	0.884*
Haemoglobin (g/dL)	12.4 (11.6–17)	11.6 (9.2–15.5)	11.65 (8.9–15.2)	0.096*
Platelet count (10^4/μL)	11.7 (5.9–58.1)	11.3 (4.8–32.4)	14.9 (7.6–40)	0.529*
PT (INR)	1.00 (0.92–1.19)	1.04 (0.93–1.67)	1.07 (0.87–1.34)	0.561*
AST (IU/L)	106 (27–352)	78 (30–258)	107 (44–464)	0.539*
ALT (IU/L)	106 (38–395)	82 (37–275)	157.5 (40–354)	0.619*
ALP (IU/L)	492 (233–1954)	479 (234–828)	636 (306–2977)	0.221*
γ-GTP (IU/L)	293 (41–1447)	107 (29–457)	122.5 (23–1417)	0.147*
Bilirubin (mg/dL)	0.9 (0.4–1.8)	0.9 (0.4–2.6)	1.25 (0.3–10.4)	0.530*
Albumin(g/dL)	3.7 (3.3–4.7)	3.8 (2.7–4.5)	3.5 (2.9–4.4)	0.329*
METAVIR score				
A 0/1/2/3	0/8/3/0	0/8/8/1	0/7/5/0	0.594†
F 0/1/2/3/4	1/8/2/0/0	1/9/7/0/0	5/5/2/0/0	0.066†
Immunosuppression				
Tacrolimus	8	16	7	0.257†
Tacrolimus + MMF	2	0	3	
Tacrolimus + prednisolone	1	0	2	
Cyclosporine	0	1	0	
Cyclosporine + MMF	0	1	0	
Trough level for tacrolimus (ng/mL)	5.9 (3.4–8.7)	5.95 (3.3–10.9)	6.4 (3.8–9.1)	0.752*

PT, prothrombin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; MMF, mycophenolate mofetil; LDLT, living donor liver transplantations; SVR, sustained virological response. Qualitative variables are shown in number; and quantitative variables expressed as median (range). *Kruskal–Wallis test, †chi-square test.

that, steroid administration was terminated. Mycophenolate mofetil (MMF) was administered to patients who experienced refractory rejection or required reduction in tacrolimus or cyclosporine doses because of adverse events.

Virological assays

Hepatitis C virus genotype was determined using a genotyping system based on polymerase chain reaction (PCR) of the core region using genotype-specific PCR primers [17]. Serum HCV RNA load was evaluated once a month during treatment and 24 weeks after treatment, using PCR and an Amplicor HCV assay (Cobas Amplicor HCV Monitor; Roche Molecular Systems, Pleasanton, CA, USA).

Statistical analysis

Wilcoxon and Kruskal–Wallis tests, chi-square tests and t-tests were used to analyse the continuous variables, categorical variables and histological changes, respectively. The Kaplan–Meier method was used to estimate the rates of patients who showed a progression of fibrosis to stage F3 or F4 after the initiation of the interferon therapy; log-rank tests were used to compare these rates among groups. Significance was defined as $P < 0.05$.

RESULTS

Characteristics of patients

Hepatitis C virus RNA concentrations and histological evidence were used to diagnose 80 patients with recurrent hepatitis C after LDLT. These patients were given one of two combination therapies: interferon and ribavirin (n = 40) or peginterferon and ribavirin (n = 40) at Kyoto University between January 2001 and April 2007. Thirty-one of the 80 patients who received the combination therapy achieved SVR (Fig. 1). Among the remaining 49 non-SVR patients,