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JOURNAL OF VIRAL HEPATITIS JVH

*Journal of Viral Hepatitis*, 2015, **22**, 94–102 doi:10.1111/jvh.12258

# Virological efficacy of combination therapy with corticosteroid and nucleoside analogue for severe acute exacerbation of chronic hepatitis B

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*Received November 2013; accepted for publication February 2014*

SUMMARY. The short-term prognosis of patients with severe acute exacerbation of chronic hepatitis B (CHB) leading to acute liver failure is extremely poor. We have reported the efficacy of corticosteroid in combination with nucleoside analogue in the early stages, but virological efficacy has not been documented. Our aim was to elucidate the virological efficacy of this approach. Thirteen patients defined as severe acute exacerbation of CHB by our uniform criteria were prospectively examined for virological responses to treatment. Nucleoside analogue and sufficient dose of corticosteroids were introduced as soon as possible after the diagnosis of severe disease. Of the 13 patients, 7 (54%) survived, 5 (38%) died and 1 (8%) received liver transplantation. The decline of HBV DNA was significant between the first 2 weeks ($P = 0.02$) and 4 weeks ($P < 0.01$). Mean reduction in HBV DNA during the first 2 weeks was 1.7 ± 0.9 log copies per mL in overall patients, 2.1 ± 0.8 in survived patients and 1.2 ± 0.9 in dead/transplanted patients. The decline of HBV DNA was significant between the first 2 weeks ($P = 0.03$) and 4 weeks ($P = 0.02$) in survived patients, but not in dead/transplanted patients. Our study shows that corticosteroid treatment in combination with nucleotide analogue has sufficient virological effect against severe acute exacerbation of CHB, and a rapid decline of HBV DNA is conspicuous in survived patients.

*Keywords:* chronic hepatitis B, corticosteroid, nucleoside analogue, severe acute exacerbation, viral reduction.

## INTRODUCTION

An estimated 350 million persons worldwide are chronically infected with hepatitis B virus (HBV) [1]. Reactivation of HBV is a well-characterized syndrome marked by the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactive or resolved HBV infection. Reactivation is often spontaneous, but can also be triggered by cancer chemotherapy, immune suppression or alteration in immune function. Acute exacerbation, which is characterized by a high alanine aminotransferase (ALT) level and jaundice, sometimes occurs and may progress to acute liver failure (ALF) and death. The short-term prognosis of patients with severe acute exacerbation of chronic hepatitis B (CHB) leading to ALF is extremely poor [2–4]. Liver transplantation has been the only definitive therapy available to salvage this group of patients. However, the problem of a shortage of donor livers still remains in Japan. Moreover, according to the most recent Japanese nationwide survey of 2009–2010, 'none' of the patients with fulminant liver failure among HBV carriers recovered without liver transplantation, and most patients had no indication for liver transplantation because of complicating malignant or nonmalignant diseases, and old age (Fig. 1) [5,6]. Thus, therapies other than transplantation must be further investigated.

In HBV infection, liver injury is considered to be induced mainly by cytotoxic T-lymphocyte-mediated cytolytic pathways in HBV-infected hepatocytes [7], and it was suggested that treating CHB patients with corticosteroid (CS) to inhibit an excessive immune response and prevent cytolysis of infected hepatocytes would be reasonable, if the HBV could be controlled [8].

Nucleoside analogues (NA), such as lamivudine (LMV), entecavir (ETV) and tenofovir (TDF), have therapeutic effects on CHB. They can markedly suppress HBV

Abbreviations: ACLF, acute-on-chronic liver failure; ALF, acute liver failure; CHB, chronic hepatitis B; CS, corticosteroid; ETV, entecavir; HBV, hepatitis B virus; LMV, lamivudine; TDF, tenofovir.

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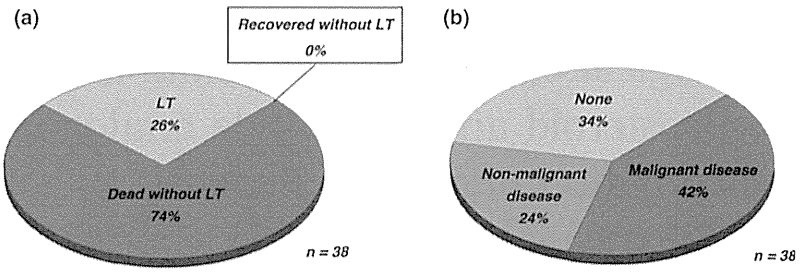


**Fig. 1** Outcome (a) and primary disease (b) of patients with fulminant hepatitis associated with HBV carrier in the Japanese nationwide survey of 2009–2010. None of the patients recovered without liver transplantation (LT), and most patients (66%) had primary disease.

replication by suppression of HBV polymerase activity. In recent studies, it has been reported that the rapid reduction in HBV DNA is a good predictor for the survival of patients of acute-on-chronic liver failure (ACLF) associated with HBV treated with NA monotherapy [9,10].

In our previous studies, we reported that the introduction of high-dose CS and NA could significantly reverse deterioration in patients with 'clinically severe, life-threatening' exacerbation of CHB compared with historical controls, when used in the early stage of illness and for more than a few weeks [11–13]. But the virological efficacy of the combination therapy with CS and NA is unknown.

In this study, we analysed patients with clinically severe acute exacerbation of CHB treated by the initiation of sufficient dosages and durations of CS and NA, to clarify the virological efficacy of the treatment.

## MATERIALS AND METHODS

### *Patients*

Thirteen patients with severe acute exacerbation of CHB admitted to our liver unit (Chiba University Hospital) between 2000 and 2012 were studied. The diagnosis of a CHB viral carrier state was made based on either the positivity of hepatitis B surface antigen (HBsAg) for at least 6 months before entry or, in patients with follow-up periods less than 6 months before entry, it was based on the positivity of HBsAg, the presence of antihepatitis B core antibody (HBcAb) at a high titre and negativity or a low titre of IgM antihepatitis B core antibody (IgM-HBc). Patients fulfilling all the following three criteria during the course were defined as having severe exacerbation: prothrombin time (PT) activity ≤60% of normal control, total bilirubin (T-Bil) ≥3.0 mg/dL and alanine transaminase (ALT) ≥300 IU/L during the course. Patients with PT activity ≤40% of control and hepatic encephalopathy were defined as having fulminant hepatitis. Patients with preexisting liver cirrhosis were excluded. All patients were in poor general condition, including general malaise, fatigue, jaundice, oedema, ascites and encephalopathy.

The work described in this manuscript was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from all patients or appropriate family members.

All patients were negative for IgM anti-HAV antibody, anti-HCV antibody, HCV RNA, IgM anti-Epstein-Barr virus antibody (IgM-EBV), IgM antiherpes simplex antibody (IgM-HSV), IgM anticytomegalovirus antibody (IgM-CMV), antinuclear antibody, antismooth muscle antibody, liver kidney microsomal antibody and antimitochondrial antibody (AMA). Patients with recent exposure to drugs and chemical agents as well as those with recent heavy alcohol intake were ruled out. One patient was HIV positive but had no clinical evidence of acquired immune deficiency syndrome.

### *Treatment protocols*

All patients treated were examined prospectively. Patients were treated with NA – LMV before 2007, ETV from 2007 and CS. Early introduction of CS was defined as follows: 40 mg or more of prednisolone (PSL) daily was administered within 10 days after the diagnosis of severe disease, using the above-mentioned criteria. This dosage was maintained for a minimum of 4 days. When the patient showed a trend towards of PT, the dosage was reduced by 10 mg at least every 4 days and tapered off. Patients for whom more than 10 days had already passed after the diagnosis were treated with delayed introduction of CS (delayed CS). Patients with marked prolongation of PT were treated with 1000 mg of methylprednisolone (MPSL) daily for 3 days followed by the same PSL therapy as that described above.

Lamivudine was administered at a daily dose of 100–300 mg (LMV group). ETV was administered at a daily dose of 0.5–1.0 mg (ETV group). Patients were also treated with intravenous glycyrrhizin, an aqueous extract of licorice root, at a daily use of 60–100 mL. This agent is reported to have anti-inflammatory activity and has been used for the treatment of acute and chronic liver injuries in Japan [14,15].

### *Serological markers*

HBsAg, hepatitis B envelope antigen (HBeAg), anti-HBe antibody (HBeAb), HBcAb, IgM-HBc and IgM anti-HAV antibody were detected by commercial radioimmunoassay (Abbott Laboratories, Chicago, IL, USA), and second- or

third-generation anti-HCV antibody was measured by enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan). IgM-EBV, IgM-CMV and IgM-HSV were examined by enzyme-linked immunosorbent assays. Antinuclear antibody, antismooth muscle antibody and AMA were examined by a fluorescent antibody method, and AMA-M2 was examined by chemiluminescent enzyme immunoassay. The HBV DNA level was measured by Amplicor monitor assay (dynamic range 2.6–7.6 logcopies per mL, Roche Diagnostics, Tokyo, Japan) or COBAS TaqMan v.2.0 (dynamic range 2.1–9.0 logcopies per mL, Roche Diagnostics).

### *Statistical analysis*

Differences in proportions among groups were compared by Fisher's exact probability test, Student's *t*-test and Welch's test.

## RESULTS

### *Clinical features of patients with severe acute exacerbation at admission*

Of the 13 patients, nine were men and four women. Mean age at the time of diagnosis was 48.9 ± 11.6 years. Five patients had primary disease and conditions (two rheumatoid arthritis, one gastrointestinal stromal tumour, one Non-Hodgkin lymphoma and one HIV positive without immunodeficiency), and four had been treated with immunosuppressive or cytotoxic drugs, suffering exacerbations after their withdrawal. Six patients were diagnosed with fulminant hepatitis on admission.

At admission to our unit, mean PT activity was 33 ± 11%, mean ALT was 968 ± 552 IU/L, and mean T-BIL was 12.6 ± 8.9 mg/dL. HBeAg/HBeAb status was +/− in 4, −/+ in 6 and +/+ in 3. Mean HBV DNA was 6.4 ± 1.7 logcopy per mL, mean alfa-fetoprotein (AFP) was 225 ± 272 ng/mL, and mean hepatocyte growth factor (HGF) was 6.5 ± 9.7 ng/mL. HBV genotype was examined in five patients, and three and two were genotype C and B, respectively. Precore/core promoter mutation was examined in nine patients, and two were wild/mutant, two mutant/wild, one mixed/wild and four mixed/mutant.

### *Type of therapies*

As initial CS, six patients received 1000 mg of MPSL, one received 500 mg of MPSL, five received 60 mg of PSL, and one received 40 mg of PSL. Mean duration between the diagnosis of severe disease and introduction of CS was 5.2 ± 4.6 days, and mean duration of CS therapy was 53.5 ± 53.1 days. Eleven patients were treated with early CS and two with delayed CS. As NA, LMV was administered to seven patients and ETV to 6. In the six patients with fulminant hepatitis, artificial liver support (plasma exchange, hemodiafiltration and transfusion of fresh frozen plasma) was performed.

### *Outcome*

Of the 13 patients, 7 (54%) survived and 5 (38%) died. The remaining one (8%), whose liver function did not recover with the combination therapy of CS and NA, underwent a liver transplantation and survived. Of the five dead patients, 4 (30%) were liver-related deaths and 1 (8%) was complication-related.

### *Biochemical responses to therapy*

Changes in PT activities, ALT levels, T-Bil levels and HBV DNA levels after the introduction of combination therapy are shown in Fig. 2.

Mean PT activity was 31 ± 9% before initiation of the combination therapy (week 0), 50 ± 24% at 2 weeks after starting (week 2) and 58 ± 25% at 4 weeks (week 4). The improvement in PT activity was significant between week 0 and 2 and between week 0 and 4 ($P = 0.03$ and $P < 0.01$, respectively). The mean ALT level was 1055 ± 606 IU/L at week 0, 112 ± 101 at week 2 and 76 ± 48 at week 4. The decline in ALT was significant between week 0 and 2 and between week 0 and 4 ($P < 0.01$, respectively). The mean T-Bil level was 13.7 ± 8.7 mg/dL at week 0, 12.0 ± 9.1 at week 2 and 10.1 ± 9.5 at week 4, changes not reaching statistical significance in the 4 weeks.

### *Virological responses to therapy*

Mean HBV DNA was 6.5 ± 1.7 log copies per mL at week 0, 4.8 ± 1.5 at week 2 and 3.6 ± 1.5 at week 4. The decline in HBV DNA was significant between week 0 and 2 and between week 0 and 4 ($P = 0.02$ and $P < 0.01$, respectively). The mean reduction in HBV DNA was 1.7 ± 0.9 log copies per mL between week 0 and 2, and 1.6 ± 1.3 log copies per mL between week 2 and 4.

### *Complication of combination therapy*

After the start of treatment for severe acute exacerbation of CHB, three patients had additional complications, one with pneumonia due to pneumocystis and cytomegalovirus, one with pneumonia due to pneumocystis and one with enteritis due to methicillin-resistant staphylococcus aureus (MRSA).

### *Comparison between survived and dead/transplanted patients*

Baseline differences in mean age, sex, ALT level, T-Bil level, PT activity, AFP, HGF and HBV DNA level were not statistically significant between survived patients and dead/

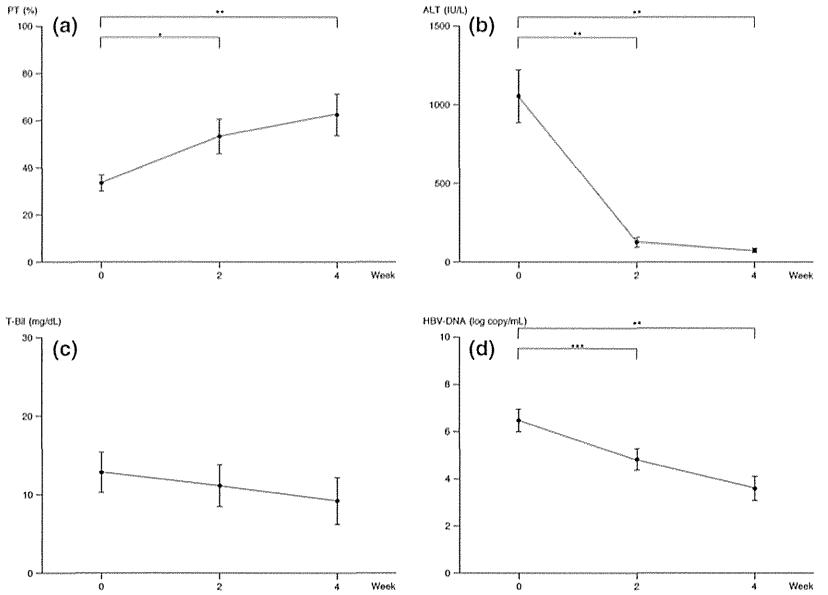


**Fig. 2** Changes in prothrombin time (PT) activities (a), alanine aminotransferase (ALT) (b), total bilirubin (T-Bil) (c) and HBV DNA level (d) in 13 patients with severe acute exacerbation of chronic hepatitis B treated with corticosteroid in combination with nucleoside analogue; $*P = 0.03$, $**P < 0.01$, $***P = 0.02$.

**Table 1** Comparison of characteristics between survived and dead/transplanted patients

| | Survived $n = 7$ | Dead/transplanted $n = 6$ | $P$ |
|---|---|---|---|
| Age (years) | 45.3 ± 10.3 | 53.2 ± 12.6 | 0.25 |
| Sex (M/F) | 6/1 | 3/3 | 0.27 |
| Fulminant hepatitis on admission | 1 | 5 | 0.03 |
| LMV/ETV | 4/3 | 3/3 | 1.00 |
| PT (%) | 36 ± 11 | 29 ± 11 | 0.28 |
| ALT (IU/L) | 1048 ± 628 | 875 ± 488 | 0.59 |
| T-Bil (mg/dL) | 12.7 ± 10.7 | 12.4 ± 7.1 | 0.97 |
| AFP (ng/mL) | 134 ± 234 | 351 ± 296 | 0.21 |
| HGF (ng/mL) | 1.9 ± 1.2 | 12.5 ± 12.3 | 0.13 |
| HBV DNA (log copies per mL) | 6.7 ± 1.6 | 6.0 ± 1.9 | 0.49 |
| HBV DNA reduction (log copies per mL) | | | |
| Week 0–2 | −2.1 ± 0.8 | −1.2 ± 0.9 | 0.16 |
| Week 2–4 | −1.4 ± 0.8 | −1.8 ± 1.8 | 0.72 |

LMV, lamivudine; ETV, entecavir; PT, prothrombin time; ALT, alanine aminotransferase; T-BIL, total bilirubin; AFP, alfa-fetoprotein; HGF, hepatocyte growth factor.

transplanted patients. The proportion of fulminant hepatitis was higher in dead/transplanted patients ($P = 0.03$) (Table 1).

Changes in PT activities, ALT levels, T-Bil levels and HBV DNA levels of both groups after the introduction of combination therapy are shown in Fig. 3. The improvement in PT activity was significant between week 0 and 2 and between week 0 and 4 ($P = 0.01$, respectively) in survived patients, but not significant in dead/transplanted patients. The decline of ALT was significant between week 0 and 2 and between week 0 and 4 in both groups ($P = 0.03$ and $P = 0.02$ in survived patients and $P < 0.01$ in dead/transplanted patients, respectively). In both groups, the changes in mean T-Bil levels were not significant at 4 weeks. The decline of HBV DNA was significant between week 0 and 2 and between week 0 and 4 ($P = 0.03$ and

$P = 0.01$, respectively) in survived patients, but was not significant in dead/transplanted patients. The mean reduction in HBV DNA was 2.1 ± 0.8 log copies per mL between week 0 and 2 and 1.4 ± 0.8 log copies per mL between week 2 and 4 in survived patients, and 1.2 ± 0.9 log copies per mL between week 0 and 2 and 1.8 ± 1.8 log copies per mL between week 2 and 4 in dead/transplanted patients. The reduction in HBV DNA was not different between week 0 and 2 in both groups.

### *Comparison between LMV and ETV groups*

Baseline differences in mean age, sex, the proportion of fulminant hepatitis, ALT level, T-Bil level, PT activity and HBV DNA level were not statistically significant between the LMV and ETV groups (Table 2).

Changes in PT activities, ALT levels, T-Bil levels and HBV DNA levels of both groups after the introduction of combination therapy are shown in Fig. 4. The improvement in PT activity was significant between week 0 and 4 ($P = 0.03$) in the ETV group, but was not significant in the LMV group. The decline in ALT was significant between week 0 and 2 and between week 0 and 4 in both groups (both $P < 0.01$ in the LMV group, $P = 0.02$ and $P = 0.01$ in the ETV group, respectively). In both groups, the changes in mean T-Bil levels were not significant at 4 weeks. The decline in HBV DNA was significant between week 0 and 4 ($P = 0.01$) in the ETV group, but was not significant in the LMV group. The mean reduction in HBV DNA was 1.4 ± 1.0 log copies per mL between week 0 and 2 and 1.3 ± 0.7 log copies per mL between week 2 and 4 in the LMV group, and 2.1 ± 0.7 log copies per mL between week 0 and 2 and 2.3 ± 2.3 log copies per mL between week 2 and 4 in the ETV group. The differences in reduction in HBV DNA were not significant between the two groups.

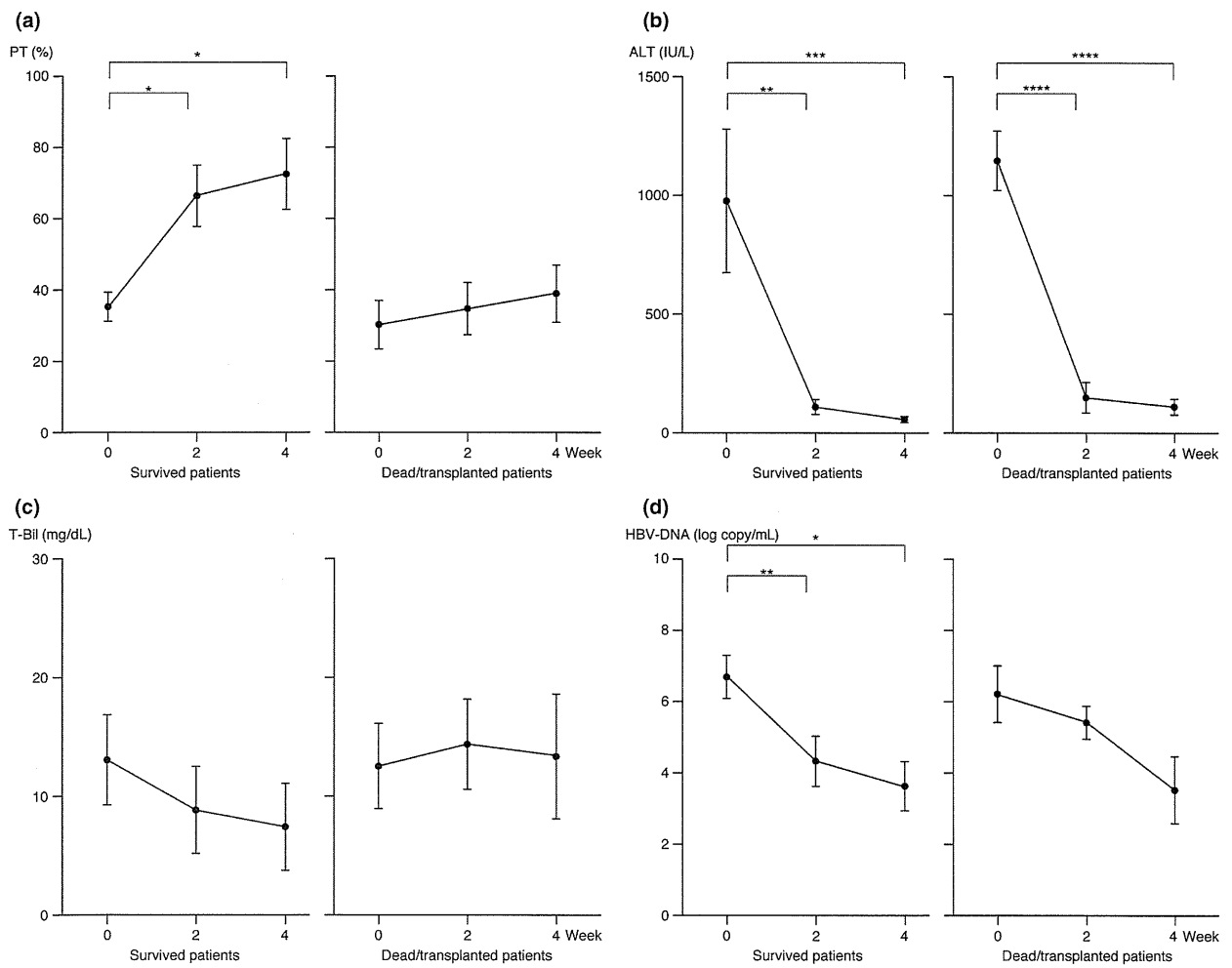


**Fig. 3** Changes in prothrombin time (PT) activities **(a)**, alanine aminotransferase (ALT) **(b)**, total bilirubin (T-Bil) **(c)** and HBV DNA level **(d)** in survived and dead/transplanted patients; *$P = 0.01$, **$P = 0.03$, ***$P = 0.02$, ****$P < 0.01$.

**Table 2** Comparison of characteristics and outcome between LMV and ETV groups

| | LMV group $n = 7$ | ETV group $n = 6$ | $P$ |
|---|---|---|---|
| Age (years) | 47.6 ± 13.7 | 50.5 ± 9.9 | 0.67 |
| Sex (M/F) | 6/1 | 3/3 | 0.27 |
| Fulminant hepatitis on admission | 4 | 2 | 0.59 |
| PT (%) | 28 ± 4 | 38 ± 15 | 0.17 |
| ALT (IU/L) | 833 ± 656 | 1126 ± 399 | 0.37 |
| T-Bil (mg/dL) | 11.5 ± 3.6 | 13.9 ± 13.0 | 0.63 |
| HBV DNA (log copies per mL) | 6.1 ± 1.7 | 6.8 ± 1.9 | 0.47 |
| HBV DNA reduction (log copies per mL) | | | |
| Week 0–2 | −1.4 ± 1.0 | −2.1 ± 0.7 | 0.25 |
| Week 2–4 | −1.3 ± 0.7 | −2.3 ± 2.3 | 0.22 |
| Outcome | | | |
| Survived | 4 | 3 | 1.00 |
| Dead/transplanted | 3 | 3 | |

LMV, lamivudine; ETV, entecavir; PT, prothrombin time; ALT, alanine aminotransferase; T-BIL, total bilirubin.

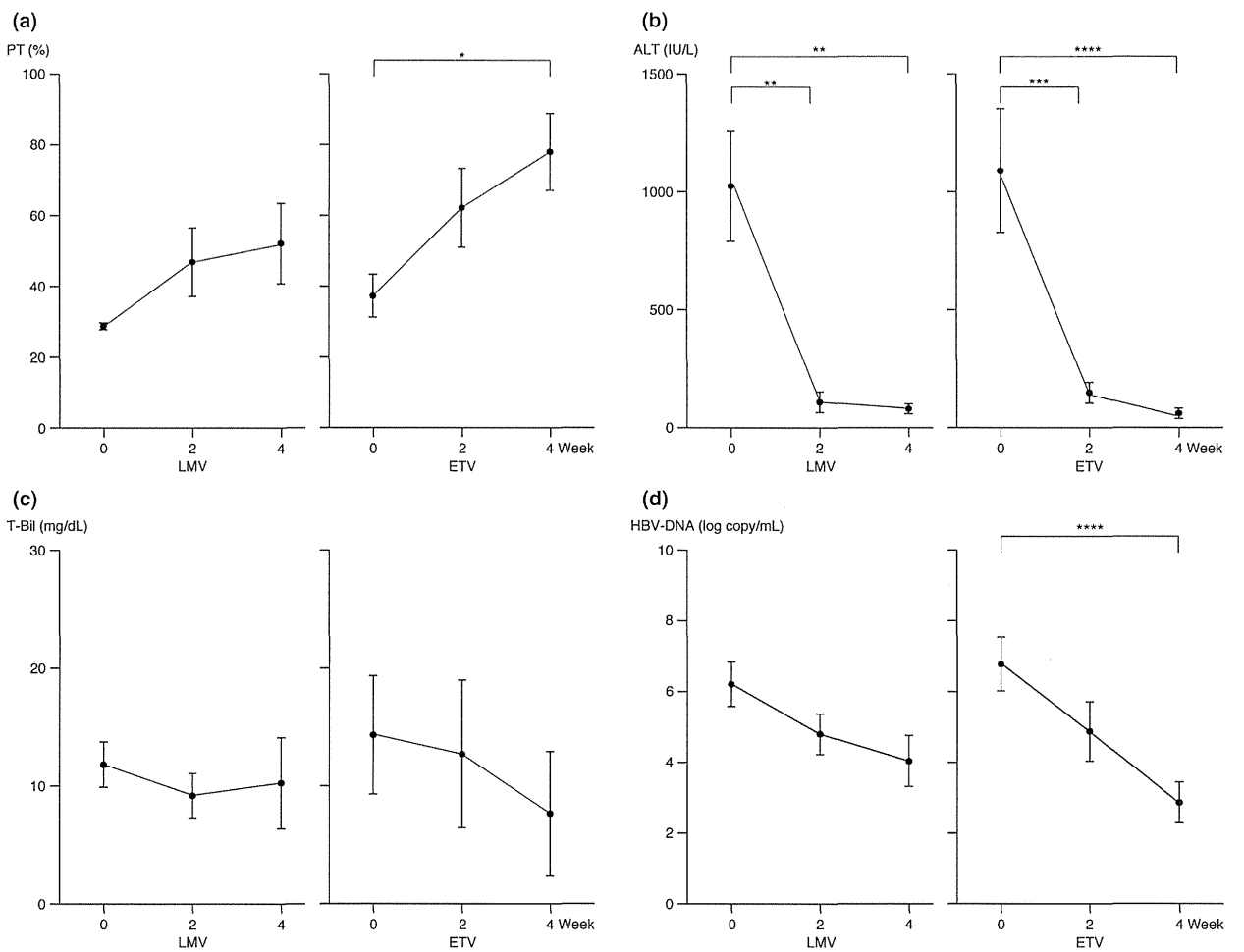


**Fig. 4** Changes in prothrombin time (PT) activities **(a)**, alanine aminotransferase (ALT) **(b)**, total bilirubin (T-Bil) **(c)** and HBV DNA level **(d)** in the LMV and ETV groups; *$P = 0.03$, **$P < 0.01$, ***$P = 0.02$, ****$P = 0.01$.

## DISCUSSION

This study shows that CS treatment in combination with NA has the sufficient virological effect against severe acute exacerbation of CHB, and the rapid decline of HBV DNA is conspicuous in survived patients.

In this study, the combination therapy with CS and NA showed a rapid decline of HBV DNA especially in survived patients. In a recent randomized controlled study that evaluated the efficacy of TDF and determine the predictor of spontaneous reactivation of CHB with ACLF, more than 2 log reduction in HBV DNA levels at 2 weeks was found to be an independent predictor of survival, with the authors concluding that the reduction in HBV DNA level at 2 weeks is a desirable goal [10]. In another retrospective study that evaluated the efficacy of LMV and determined the predictor of CHB with ACLF, >2-log reduction at 4 weeks was found to be a good predictor of outcome [9]. The rapid decline of HBV DNA is one of the important factors for recovery. In our present study, the combination therapy with CS and NA achieved a desirable goal at week 2 in survived patients.

On the other hand, in a recent study from China that evaluated the efficacy of NA treatment with HBV-associated ACLF, LMV and ETV achieved significant viral suppression after 3 months, but did not improve survival [16]. Recently, NAs have been administered in severe reactivation of CHB. In one initial case series from Japan, three patients with cirrhosis who presented with severe acute exacerbation and hepatic encephalopathy responded dramatically to LMV treatment [17]. However, later studies did not demonstrate any benefit of LMV treatment for survival [3,4]. In the management of severe acute exacerbation of CHB, the rapid decline of HBV DNA is one of the important goals, but it is not sufficient to improve survival. It is reported that HBV DNA decreases rapidly with the administration of NAs, but improvements in liver function and liver regeneration are delayed by a few weeks to a few months [11,18,19]. During this time-lag phase, excessive immunological reaction may continue, liver cell injury may progress and liver regeneration may be impaired. Therefore, it is understood that additional rapid cessation of ongoing necro-inflammation is essential for the achievement of liver regeneration.

We have used CSs for the rapid cessation of necro-inflammation. In severe acute exacerbation of CHB, liver injury is considered to be induced mainly by cytotoxic T-lymphocyte-mediated cytolytic pathways of infected hepatocytes [7], and it has been suggested that treating CHB patients with CSs to inhibit an excessive immune response and prevent cytolysis of infected hepatocytes would be reasonable, if the HBV could be controlled [8]. Our present study showed that HBV is controllable in patients treated with immunosuppressive therapy. In a recent study from China that evaluated the combination therapy with short-term dexamethasone and LMV for pre-ACLF patients, no significant differences in HBV DNA levels were observed between the dexamethasone group and control group during the observation period [20]. In our previous studies, we reported that the early introduction of high-dose CS improve survival [11], the combination therapy with high-dose CS and NA could reverse deterioration of severe acute exacerbation of CHB [12] and that more than a few weeks of CS treatment in combination with NAs is required [13]. Additionally, we recently reported that the introduction of high-dose CS in the early stage of viral ALF suppressed the destruction of hepatocytes [21]. In another study from Japan evaluating the predictors of progression to hepatic decompensation during severe acute exacerbation of CHB, the authors concluded that antiviral therapies with CS should be started as soon as possible in cases with high T-Bil level and/or low PT levels [22]. Moreover, in a recent meta-analysis evaluating the safety, efficacy and side effects of glucocorticoid therapy for severe viral hepatitis B, treatment with glucocorticoids significantly increased the survival rate of patients with severe viral hepatitis B [23,24]. We believe that both rapid decline of HBV DNA and cessation of necro-inflammation are necessary to improve the survival of severe acute exacerbation of CHB, and the combination therapy of CS and NA is a reasonable strategy.

The decline of HBV DNA could be brought not only by NA but also the host immune response. In the randomized study of ACLF patients described above [10], the nine of fourteen patients had >2 log reduction in the HBV DNA level in the TDF group, otherwise none of nine patients had >2 log reduction in the placebo group at day 15. In another study [16], patients treated with NAs had significant reduced HBV DNA levels at weeks 2, 4, 6, 8, 10 and 12 compared with patients without NAs. Thus, NAs bring the decline of HBV DNA more effectively than the host immune response alone.

In a study from Hong Kong, ETV was associated with increased short-term mortality compared with LMV although the patients treated with ETV had superior virological response compared to those on LMV [25]. The cause of increased short-term mortality was unknown. In the present study, the recovery rate of patients treated with ETV was not different from that with LMV, and ETV-treated patients had significant reduction in HBV DNA at week 4. ETV is a potent HBV inhibitor with a high barrier to resistance and can therefore be confidently used as a first-line monotherapy for CHB [26].

The prognosis of patients with severe acute exacerbation of CHB leading to ALF is extremely poor. In the recent studies, Cui, *et al.* [16] reported that the survival at 3 months of HBV-associated ACLF was 49.25% for patients with NA treatment and 40.54% without NA treatment, and Garg, *et al.* [10] reported that the survival at 3 months of severe spontaneous reactivation of CHB presenting as ACLF was 57% for patients with TDF treatment and 15% with placebo. In the present study, overall survival of our patients

was 54%, which is equal to those with NA treatment studies described above, but the proportion of fulminant liver failure at admission was 46% in our study which was higher than those in two studies described above (21% ($P = 0.08$) and 7% ($P = 0.03$), respectively). This clearly means that our patients had severer disease than those in two studies. Therefore, we suppose that the survival by combination therapy with CS and NA is not inferior to that by NA monotherapy, although we could not include placebo-controlled patients, considering the current knowledge of the poor prognosis of the patients. Almost our 'dead/transplanted' patients had already developed fulminant liver failure at admission to our units, the state of impaired liver regeneration. Therefore, we have administered combination therapy with early CS and NA according to the appropriate definition of severe disease before the development into fulminant liver failure.

Regarding adverse events, opportunistic infections occurred in three of our patients with combination therapy. These opportunistic infections seem to be specific complications of immunosuppressive therapy and the immunodeficient status of ALF. Therefore, the appropriate definition of severe acute exacerbation of CHB is required to decide the indication for combination therapy.

Our study had a few limitations. First, the number of patients in our study was small. Second, this was not randomized study. Severe acute exacerbation of CHB is an uncommon but potentially life-threatening condition. Ethical issues obviously prevent a randomized control study with such life-threatened patients. Further multicenter studies are necessary.

In summary, our study showed that early introduction of CS treatment in combination with NA has sufficient virological effect against severe acute exacerbation of CHB, and the rapid decline of HBV DNA is distinct in survived patients. The survival in our patients by combination therapy with CS and NA is not inferior to that in recent reports by NA monotherapy considering the severity of our patients. We believe that both rapid decline of HBV DNA and cessation of necro-inflammation are necessary to improve the survival of severe acute exacerbation of CHB. For this purpose, 'high-dose' CS in combination with NA should be administered 'as soon as possible' according to the 'appropriate definition' of severe disease.

## ACKNOWLEDGEMENTS

We are indebted to all our colleagues at the liver units of our hospitals who cared for the patients described herein. This study was supported in part by a Health Labour Sciences Research Grant from the Ministry of Health, Labor and Welfare of Japan as a project by the Intractable Hepato-Biliary Disease Study Group of Japan.

## DISCLOSURES

All authors have nothing to disclose.

## CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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CORRESPONDENCE

# Reappearance of serum HBV DNA in patients with hepatitis B surface antigen seroclearance

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**Key words:** HBV DNA; reactivation; corticosteroid; CD20; HBsAg.

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To the Editor:

We read with great interest the article by Di Bisceglie et al.[1] In this report from the United States, the recommendation that all patients undergoing chemotherapy, immunosuppressive therapy, hematopoietic stem cell transplantation or solid organ transplantation be screened for active or prior HBV infection by testing for HBsAg and anti-HBc in serum. This problem is also serious in Japan, where no universal vaccination programs against HBV exist, and HBV infections are still viewed as important issues.[2,3]

The elimination of HBsAg is one of the goals in the treatment of HBV infection. We examined the incidence of the reappearance of HBV DNA in chronic hepatitis B patients. We compared the backgrounds of 9 patients who achieved HBsAg seroclearance treated by nucleoside analogues (NAs group) with those of 13 patients in whom natural HBsAg seroclearance occurred (control group). We also evaluated HBV DNA levels at 4-12-month intervals after the disappearance of HBsAg. HBV DNA reappearance was defined as the detection of serum HBV DNA after the disappearance of both of HBV DNA and HBsAg. Age at HBsAg seroclearance in the NAs group and in the control group were 62±14 and 59±6.3 years, respectively. In the NAs group, 5 patients (56%) were treated with immunosuppressive agents (3, antibodies to CD20

[rituximab]; 1, corticosteroid; and 1, tacrolimus; p = 0.011, compared with the control group [0%]). After HBsAg seroclearance, HBV DNA reappeared in 3 (33%) and 2 (15%) individuals in the NAs and control groups, respectively (Figure). Of interest, patients in whom HBV DNA reappeared after HBsAg seroclearance did not exhibit any elevation in ALT or the reappearance of HBsAg. There was no significant difference in patients' characteristics between patients with and patients without the reappearance of HBV DNA in either the NAs or control group.

HBV DNA reappearance was occasionally observed in chronic hepatitis B patients with HBsAg seroclearance, suggesting that reactivation occurs in patients who have recovered from hepatitis B and have anti-HBc but no detectable serum HBsAg.[1] Measurement of HBV DNA after HBsAg seroclearance may thus also be important in such patients receiving chemotherapy. Our data strongly support their recommendations.[1] Further understanding of the mechanism of HBV reactivation with or without immunosuppressant or anti-cancer drug treatment is needed.[1,3]

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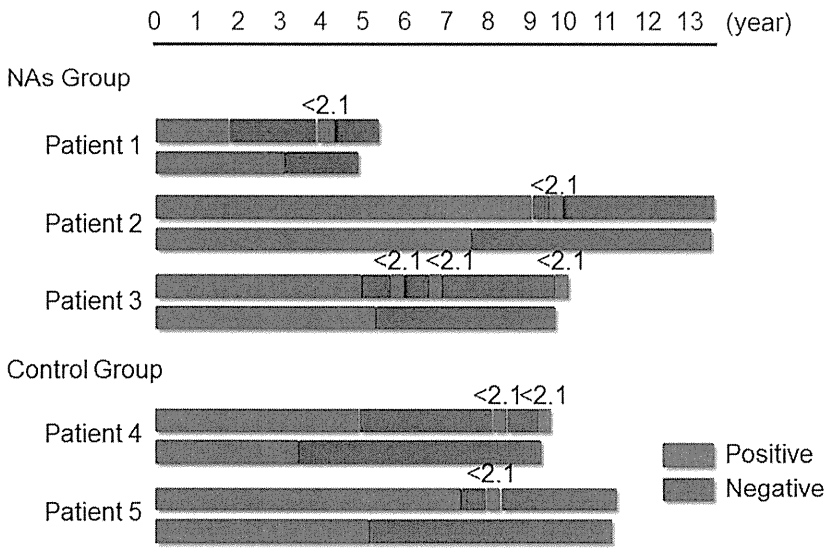
**Figure. Patients with reappearance of HBV DNA after HBsAg-seroclearance.**

In each patient, upper and lower bars indicate HBV DNA and HBsAg, respectively.



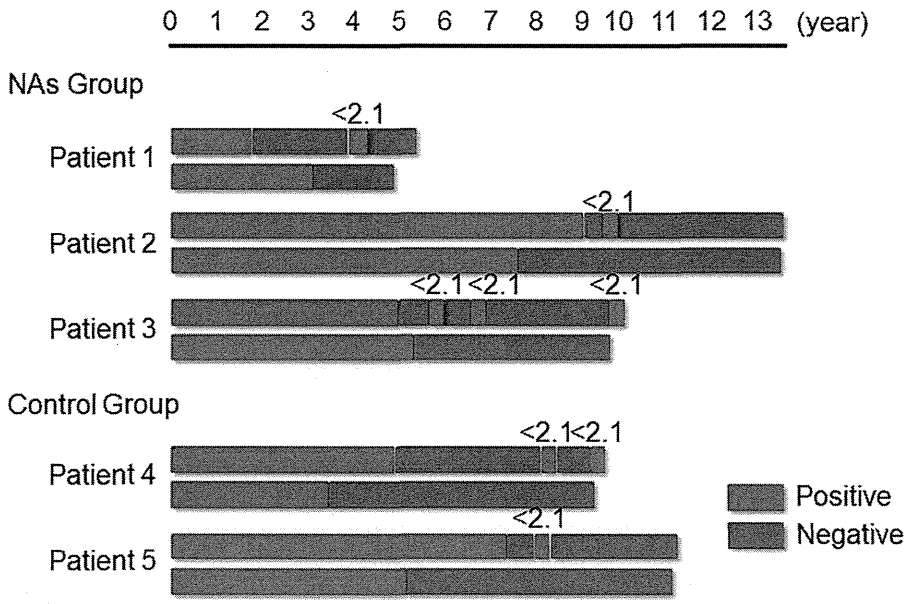



Figure. Patients with reappearance of HBV DNA after HBsAg-seroclearance.
In each patient, upper and lower bars indicate HBV DNA and HBsAg, respectively.
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J Gastroenterol (2014) 49:173–184
DOI 10.1007/s00535-013-0909-8

The Japanese Society of Gastroenterology

REVIEW

# The role of microRNAs in hepatocarcinogenesis: current knowledge and future prospects

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Received: 17 October 2013 / Accepted: 4 November 2013 / Published online: 21 November 2013


**Abstract** MicroRNAs (miRNAs) are small, noncoding RNA molecules that regulate gene expression post-transcriptionally through complementary base pairing with thousands of messenger RNAs. Although the precise biological functions of individual miRNAs are still unknown, miRNAs are speculated to play important roles in diverse biological processes through fine regulation of their target gene expression. A growing body of data indicates the deregulation of miRNAs during hepatocarcinogenesis. In this review, we summarize recent findings regarding deregulated miRNA expression and their possible target genes in hepatocarcinogenesis, with emphasis on inflammation-related hepatocarcinogenesis. Because miRNA-based strategies are being applied to clinical therapeutics, precise knowledge of miRNA functions is crucial both scientifically and clinically. We discuss the current open questions from these points of view, which must be clarified in the near future.

**Keywords** MicroRNA · Hepatocarcinogenesis · Inflammation

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## Introduction

MicroRNAs (miRNAs) are short, single-stranded, noncoding RNAs, which are expressed in most organisms, from plants to vertebrates [1]. Since the discovery of the miRNA lin-4 in *Caenorhabditis elegans* [2, 3], 1,872 miRNA precursors and 2,578 mature miRNA sequences in humans have been deposited in miRBase, a public repository hosted by the Sanger Institute, as of November 2013 [4]. Bioinformatic predictions suggest that miRNAs regulate more than 30 % of human protein-coding genes [5–7]. Through the regulation of gene expression, miRNAs are involved in various physiological and pathological processes, including cell proliferation, apoptosis, differentiation, metabolism, oncogenesis and oncogenic suppression [8, 9]. Thus, it is not surprising that deregulation of miRNAs is linked closely to various human pathological conditions. In this review, we will describe the crucial role of miRNAs in liver carcinogenesis, especially inflammation-related hepatocarcinogenesis.

## Biogenesis and functions of miRNAs

Transcription is the first step in miRNA expression (Fig. 1). Similar to most protein-coding genes, transcriptional factors, enhancers and silencers are involved in miRNA transcription [10–12]. Epigenetic mechanisms, such as promoter methylation or histone modification, also regulate miRNA transcription, and it was shown that histone deacetylase (HDAC) inhibition results in transcriptional changes in ~40 % of miRNAs [13].

Primary miRNAs, which possess stem-loop structures, are transcribed by RNA polymerase II [8]. These pri-miRNAs are processed by a microprocessor complex

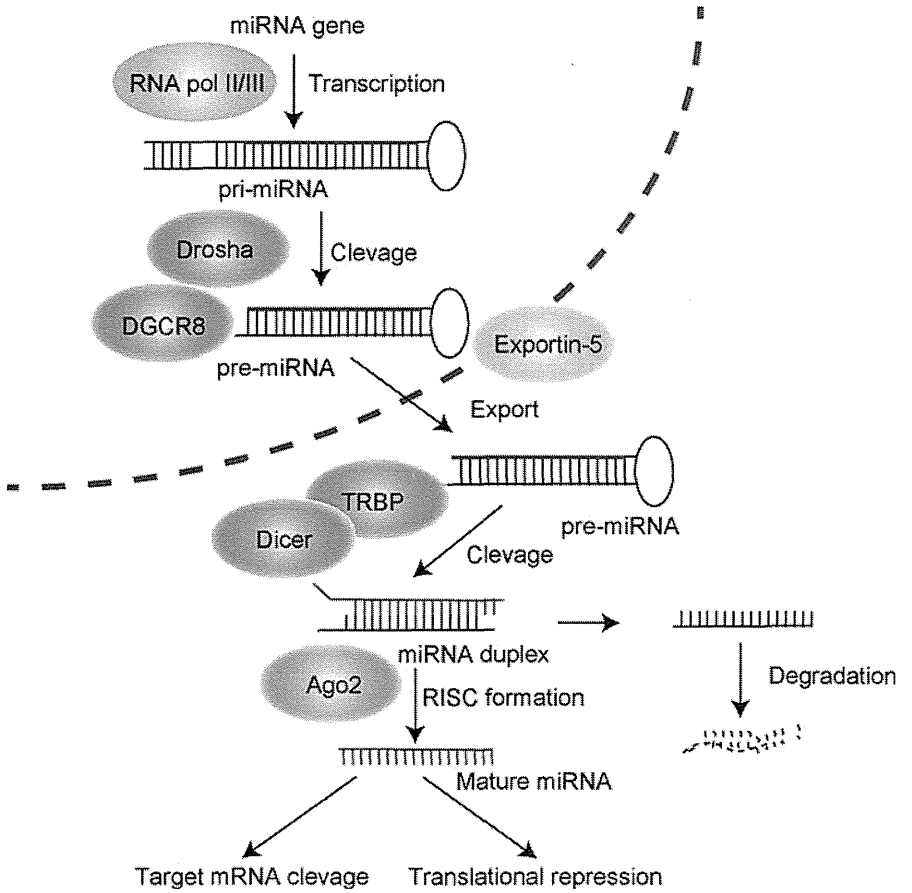


**Fig. 1** Biogenesis of miRNAs. The primary miRNA transcript (pri-miRNA) is transcribed from the genome by RNA polymerase II or III. The microprocessor complex Drosha–DGCR8 cleaves the pri-miRNA into the precursor hairpin, pre-miRNA in the nucleus. The pre-miRNA is exported from the nucleus by exportin-5–Ran-GTP. In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein, TRBP, cleaves the pre-miRNA hairpin to its mature length. The functional strand of the mature miRNA is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage or translational repression. The passenger strand (*black*) is degraded

comprising Drosha (RNAase III) [14] and DGCR8/Pasha [15] in the nucleus [16]. The processed products are approximately 65-nucleotide hairpin-shaped precursors (pre-miRNAs) that are transported to the cytoplasm via exportin-5 [17, 18]. Pre-miRNAs are further cleaved into mature miRNAs by Drosha and Dicer RNA polymerase III. Mature miRNA duplexes are loaded onto an RNA-induced silencing complex (RISC) and are unwound into the single-stranded mature form [19–21]. The resulting co-complex directly targets the 3′-untranslated regions (3′-UTRs) of target mRNAs, depending on the sequence similarities, to negatively regulate their expression by enhancing mRNA cleavage or inhibiting translation (Fig. 1) [8, 22]. Because most miRNAs guide the recognition of imperfect matches of target mRNAs, individual miRNAs have multiple (probably hundreds) of mRNA targets. In addition, multiple miRNAs can cooperate to regulate the expression of the same transcript [6]. Thus, depending upon the identity of the target mRNAs, miRNAs play roles as "fine-tuners of gene expression" in the control of various biological functions.

Identifying functionally important miRNA target genes is crucial for understanding the impact of specific miRNAs on cellular function. However, this is challenging because miRNAs usually have imperfect complementarity with their targets [22]. In mammals, the most consistent requirement for miRNA-target interaction, although not always essential, is a contiguous and perfect pairing of the miRNA (nt 2–8), representing the "seed" sequence [22]. In many cases, the seed sequences determine this recognition, but in other cases, additional determinants are required, such as reasonable complementarity to the miRNA 3′ half to stabilize the interaction. In addition, target pairing to the center of some miRNAs has also been reported [23]. Although public miRNA target prediction algorithms, such as TargetScan [24] and PicTar [25], have facilitated the rapid identification of miRNA target genes [22], candidates should be validated experimentally.

## miRNAs and cancer

The involvement of miRNAs in cancer pathogenesis is well established. miRNAs can affect six hallmarks of malignant cells, which are (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) angiogenesis, and (6) invasion and metastasis [26]. miRNAs are frequently

up- or downregulated in malignant tissues and can be considered oncogenes or tumor suppressors, respectively. However, it is essential to test experimentally whether the deregulated miRNAs are actually causative to carcinogenesis, since miRNAs have a very restricted tissue-specific expression and the apparent miRNA modulation in cancer tissues may only reflect the different constituents of a cell population as compared to normal tissues. Extensive analyses have confirmed the causative roles of miRNAs in cancer by using either human cancer cells or genetically engineered animal models, such as transgenic expression of miR-155, miR-21 and miR-15-a/16-1, which are sufficient to initiate lymphomagenesis in mice [27–29]. These results suggest the potential role of miRNAs in the pathogenesis of carcinogenesis and as therapeutic targets.

## miRNAs and hepatocarcinogenesis

Numerous reports regarding the deregulated expression of miRNAs in human hepatocellular carcinoma (HCC) are extant. Most studies compared the miRNA expression levels between cancer tissues and background non-tumorous tissues, selected candidate miRNA(s) and revealed their target genes, which may be involved in carcinogenesis. As shown in Tables 1 and 2, many miRNAs have been identified as downregulated or upregulated in recent studies (Tables 1, 2). However, these numerous results are not always superimposable due to the large variances in the results. These significant differences may be due to several reasons, such as the use of different techniques or different samples as controls, normal liver tissues versus peritumoral non-neoplastic tissues. In addition, one may need to take into consideration the fact that HCCs arise in background livers with different etiologies, such as hepatitis B, hepatitis C or steatohepatitis, and also the age or sex of the tissue-derived patients and background liver condition, such as fibrosis staging or inflammation activity, which may result in differences in the expression status of miRNAs. Despite these considerable limitations, the list suggests that diverse miRNAs play crucial roles in hepatocarcinogenesis. We will briefly describe some of them below.

The expression levels of miRNAs have restricted tissue specificities. In the liver, miR-122, miR-192 and miR-199a/b-3p are the three most expressed miRNAs, accounting for 52, 17 and 5 % of all mRNAs in the tissues, respectively [30]. The tumorigenic role of the loss of miR-122 was confirmed in gene-knockout mice [31, 32] and its expression is indeed decreased in half of the HCCs, especially non-viral HCCs [30]. We also reported that decreased expression of miR-122 is linked with poor prognosis of HCC [33]. While miR-192 does not appear to be deregulated in HCC samples in previous studies, miR-199a/b-3p is decreased with high frequency in HCC, which is closely linked to a poor prognosis of HCC [30]. In contrast, miR-21, whose expression is increased following rat hepatectomy [34], is upregulated as a known oncomiRNA and represses PTEN signaling, resulting in promotion of HCC development [35]. Although individual miRNAs may be involved in hepatocarcinogenesis, because miRNAs often function co-operatively, the extent of their involvement remains to be determined.

As described above, miRNAs usually have multiple mRNA targets. Thus, it is not practical to describe only a few genes as being responsible for the phenotypes by deregulation of specific miRNAs, while many studies identify specific genes as targets of specific miRNAs. Nonetheless, the identified targeted genes are generally related to at least one of the hallmarks of cancer, such as cell growth, apoptosis, invasion, and so on. These results suggest that the deregulation of miRNA expression might mediate hepatocarcinogenesis through deregulating the expression of their target genes.

The miRNAs identified as deregulated in hepatocarcinogenesis may be useful as diagnostic and prognostic markers [36], because miRNAs in the circulation are reported to be relatively stable [37]. Also, deregulated miRNAs may be candidate therapeutic and preventive targets against HCC. However, to include the obtained results in clinical interventional applications, it is necessary to confirm if the deregulated miRNAs are truly drivers or are simply passive in hepatocarcinogenesis. To this end, genetically modified mice may provide some information. In addition, to correctly interpret the data, a standard method of normalizing the microRNAome data between studies may also be crucial. Since there are multiple target genes of miRNAs and, conversely, one transcript can be targeted by multiple miRNAs, a more systematic comparison using miRNA data, transcriptome data and proteome data would increase our understanding of the consequences of the deregulation of miRNAs during hepatocarcinogenesis. From this point of view, systematic and comprehensive target gene analyses for in silico systems biology models may be one option to resolve these issues.

## miRNAs linked to inflammation-mediated hepatocarcinogenesis

Inflammation is considered to be a major cause of cancer [38, 39]. In the liver, hepatocarcinogenesis frequently occurs in persistently inflamed liver tissues caused by chronic hepatitis viral infection or non-alcoholic steatohepatitis. However, the molecular linkage between chronic inflammation and carcinogenesis is not well characterized.