Thymalfasin, a promising adjuvant therapy in small hepatocellular carcinoma after liver resection

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Abstract
There is limited information available concerning the effect of thymalfasin (Ta1) as an adjuvant therapy in hepatocellular carcinoma (HCC) patient who received liver resection. The present study aimed to evaluate whether Ta1 can improve the prognosis of small HCC patients after liver resection.

A total of 206 patients with small HCC who underwent liver resection were analyzed in our retrospective cohort study. Patients were divided into 2 groups: group A (resection + Ta1, n = 44) and group B (resection, n = 162). Clinical data, overall survival (OS), and recurrence-free survival (RFS) were compared. Prognostic factors were identified using multivariate analysis.

After a median follow-up of 47.0 months, 134 patients (65%) had recurrence, and 62 patients (30.09%) died. The 1, 3, and 5-year OS rate of patients in group A was 97.7%, 90.6%, and 82.9%, respectively, and 95.1%, 80.5%, and 62.9%, respectively, for patients in group B (P = .014). The 1, 3, and 5-year RFS rate of patients in group A was 70.5%, 56.8%, and 53.3%, respectively, and 65.8%, 41.3%, and 32.1%, respectively, for patients in group B (P = .015). Multivariate analysis indicated that Ta1 was an independent prognostic factor for both OS (P = .015, hazard ratio 0.349, 95% confidence interval 0.149–0.816) and RFS (P = .019, hazard ratio 0.564, 95% confidence interval 0.349–0.910).

Ta1 as an adjuvant therapy after liver resection may improve the prognosis of small HCC patients after liver resection.

Abbreviations: HBV = hepatitis B virus, HCC = hepatocellular carcinoma, MVI = microvascular invasion, NLR = neutrophil to lymphocyte ratio, OS = overall survival, RFS = recurrence-free survival, Ta1 = thymalfasin, TACE = transarterial chemoembolization.

Keywords: hepatocellular carcinoma, liver resection, Milan criteria, prognostic factor, thymalfasin

1. Introduction
Hepatocellular carcinoma (HCC) is 5th common malignancies and the 3rd-leading cause of cancer-related death worldwide. Because of high hepatitis B virus (HBV) infection, China alone accounts for about 50% of the total number of cases and deaths.[1,2] Although surgical techniques and perioperative management have improved, survival remains very poor in HCC patients. Therefore, continuing efforts to explore new approaches to impede recurrence and improve the outcome of HCC is necessary. Immunotherapy has been shown to have potential benefit but evidence is not strong enough.[3,4] Thymalfasin (thymosin α1, Ta1, commercial name: ZADAXIN) is a naturally occurring thymic polypeptide of 28 amino acids.[5]

The mechanism of this drug is related to its immune-modulating activities, and Ta1 has been widely used in various diseases, including some infection disease and malignances.[6] Previous investigations have shown that Ta1 may improve the outcomes of HCC patients who underwent transarterial chemoembolization (TACE).1,7,8 However, whether Ta1 can improve the prognosis of small HCC patients who received liver resection has not been confirmed and the underlying mechanisms remains unclear. The present study was designed to evaluate the efficacy of Ta1 as an adjuvant therapy in patients with small HCC who underwent liver resection.

2. Methods
2.1. Patients
This study was approved by the Ethics Committee of West China Hospital, Sichuan University. The HCC patients who underwent liver resection in the Department of Liver Surgery and Liver Transplantation Center of West China Hospital between February 2007 and February 2013 were identified from our prospectively maintained database. During the period, 283 small HCC patients (within Milan criteria) received curative resection. The present study aimed to evaluate whether Ta1 can improve the prognosis of small HCC patients after liver resection.
as follows: primary HBV-related small HCC (solitary tumor <5 cm in diameter or ≤3 nodules, each of them <3 cm in diameter, without major vascular invasion or distant metastasis); receiving liver resection as initial therapy in our hospital; and liver reserve function Child–Pugh grade A. Exclusion criteria included the following: extra-hepatic malignancies; previous resection, TACE, ablative therapies, or liver transplantation; loss to follow-up within 3 months after liver resection; poor liver reserve function with a Child–Pugh grade B or C; rupture of HCC; coinfection with hepatitis C virus; simultaneous splenectomy; and previously treated with To1. Based on our inclusion and exclusion criteria, 77 patients were excluded. Excluded patients were as follows: 8 had recurrent HCC, 9 had a preoperative Child–Pugh grade of B, 4 had a rupture of HCC, 5 had a history of therapy before resection, 8 lost to follow-up within 3 months after operation, 4 coinfeected with hepatitis C virus, and 39 had poor data integrity. Finally, a total of 206 patients were included in the analysis. Patients were divided into 2 groups based on whether they received To1 adjuvant therapy: group A (liver resection plus To1, n = 44), group b (liver resection only, n = 162). Details about patient selection are shown in Fig. 1. Current regimen of To1 (ZADAXIN): 1.6 mg subcutaneously twice per week for at least 6 months in the 1st half year after surgery, every 3 months during the following 3 years, and every 6 months in the 2nd, 3rd, and 4th follow-up visit minus NLR0. Data of NLR and 128 (62.1%) had a nodule 3 to 5 cm in diameter. A total of 174 patients (86.4%) had 1 nodule, and 32 (15.5%) patients had 2 or 3 nodules; 78 patients (37.9%) had a nodule <3 cm in diameter, and 128 (62.1%) had a nodule 3 to 5 cm in diameter. A total of 178 (86.4%) patients had cirrhosis, 108 (52.4%) patients had HBV-DNA levels higher than 10^3 IU/L. Neutrophil to lymphocyte ratio (NLR) was defined as absolute neutrophil counts divided by lymphocyte counts. For group A, NLR0 was defined as NLR in the first follow-up visit. AS for group B, NLR0 was defined as NLR before administration of To1. dNLR1, dNLR2, and dNLR3 were defined as the NLR of 2nd, 3rd, and 4th follow-up visit minus NLR0. Data of NLR were excluded if there was clinical symptoms or signs of sepsis at the time of blood sampling for NLR, or white blood cell counts >10^9/L.

### 3. Results

#### 3.1. Baseline characteristics

In the present research, a total of 206 patients were included in the analysis. As described in Table 1. There were 178 males (86.4%) and 28 females (14.6%), with the mean age of 50.2 ± 11.8 (range from 21 to 78) year. The median follow-up time 47.0 months (range from 21.00 to 78.00). A total of 174 patients (84.5%) had 1 nodule, and 32 (15.5%) patients had 2 or 3 nodules; 78 patients (37.9%) had a nodule <3 cm in diameter, and 128 (62.1%) had a nodule 3 to 5 cm in diameter. A total of 178 (86.4%) patients had cirrhosis, 108 (52.4%) patients had HBV-DNA levels higher than 10^3 IU/L. The baseline characteristics of patients in the 2 groups are described in Table 1. There was no significant difference between the 2 groups in the baseline characteristics and the number of patients who received adjuvant therapy after recurrence (Table 2). In group A, all of the 44 patients were administrated To1 with a median treatment time of 33.9 months (range from 6 to 93 months).

#### 3.2. Impact of To1 on OS

After a median follow-up of 47.0 months, 134 patients (65%) had recurrence, and 62 patients (30.1%) died. The 1, 3, and 5-year OS rate of all 206 patients was 95.6, 82.7, and 67.0%, respectively. When dividing the patients into 2 groups by To1, the 1, 3, and 5-year OS rate of patients in group A was 97.7, 90.6, and 82.9%, respectively, and 95.1, 80.5, and 62.9%, respectively, for patients in group B. The median OS time was 94.8 months (95% CI 85.7–103.8) for group A, 74.9 months (95% CI 68.2–81.7) for group B (log-rank test, P = .014, Fig. 2).
3.3. Impact of Ta1 on RFS

The 1, 3, and 5-year RFS rate of all 206 patients was 67.8%, 44.7%, and 36.5%, respectively. When dividing the patients into 2 groups by Ta1, the 1, 3, and 5-year RFS rate of patients in group A was 70.5%, 56.8%, and 53.3%, respectively, and 65.8%, 41.3%, and 32.1%, respectively, for patients in group B (P = .015). The median RFS time was 63.4 months (95%CI 49.3–77.6) for group A, 39.6 months (95%CI 33.3–46.0) for group B (log-rank test, P = .015 Fig. 3).

3.4. Impact of Ta1 on dynamic NLR change

Because of lacking the integrity of the follow-up blood cell data or patients having clinical symptoms or signs of sepsis at the time of blood sampling for NLR, patient number has changed in the analysis of dynamic NLR change. In group A, dNLR1 increased in 4 patients (3.5%) and decreased in 31 patients (64.6%), in group B, 109 patients (96.5%) increased and 17 patients (35.4%) decreased. dNLR3 increased in 4 (4.3%) patients, decreased in 31 (68.9%) patients in group A, increased in 10 (96.5%), decreased

Table 1

Demographic and clinical data of 206 small HCC patients according to thymalfasin.

| Factors                      | Group A (n=44) | Group B (n=162) | P     |
|------------------------------|----------------|-----------------|-------|
| Gender (male/female)         | 39/5           | 139/23          | .805  |
| Age, y                       | 49.8±11.9      | 51.7±11.6       | .342  |
| TBL, μmol/L                  | 16.2±8.2       | 15.5±6.7        | .974  |
| ALB, g/L                     | 42.6±4.4       | 41.8±4.4        | .300  |
| PT, s                        | 11.8±1.0       | 12.1±1.2        | .096  |
| Tumor size, cm, n, %         |                |                 |       |
| <3                           | 19 (43.2)      | 59 (36.4)       | .484  |
| 3–5                          | 25 (56.8)      | 103 (63.6)      |       |
| Tumor number, n, %           |                |                 |       |
| 1                            | 37 (18.0)      | 137 (66.5)      | 1.000 |
| 2–3                          | 7 (3.4)        | 25 (12.1)       |       |
| MVI, n, %                    |                |                 |       |
| Yes                          | 10 (22.7)      | 23 (13.9)       | .167  |
| No                           | 34 (77.3)      | 142 (86.1)      |       |
| Differentiation, n, %        |                |                 |       |
| High                         | 2 (4.5)        | 15 (9.3)        | .554  |
| Moderate                     | 41 (93.2)      | 14 (87.0)       |       |
| Low                          | 1 (2.3)        | 6 (88.3)        |       |
| Cirrhosis, n, %              |                |                 |       |
| Yes                          | 36 (81.8)      | 142 (87.7)      | .326  |
| No                           | 8 (18.2)       | 20 (12.3)       |       |
| HBV-eAg, n, %                |                |                 |       |
| Positive                     | 6 (13.6)       | 41 (25.3)       | .110  |
| Negative                     | 38 (86.4)      | 121 (74.7)      |       |
| AFP, ng/mL, n, %             |                |                 |       |
| ≥400                         | 9 (20.5)       | 54 (33.1)       | .139  |
| <400                         | 35 (79.5)      | 109 (66.9)      |       |
| Transfusion, n, %            |                |                 |       |
| Yes                          | 3 (6.8)        | 26 (16.0)       | .146  |
| No                           | 41 (93.2)      | 134 (84.0)      |       |
| Operation duration, min      | 224.1±51.5     | 219.8±63.9      | .703  |
| HBV-DNA                     |                |                 |       |
| <10^3 IU/mL                  | 26 (59.1)      | 72 (44.4)       | .085  |
| ≥10^3 IU/mL                  | 18 (40.9)      | 90 (55.6)       |       |
| Preoperative antivirus therapy|               |                 |       |
| Yes                          | 7 (15.9)       | 11 (6.8)        | .072  |
| No                           | 37 (84.1)      | 151 (93.2)      |       |

APP = alpha fetoprotein, ALB = albumin, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, MVI = microvascular invasion, PT = prothrombin time, TBL = total bilirubin.

Table 2

Adjuvant therapy of 206 small HCC patients according to thymalfasin after liver resection.

| Therapy                        | Group A (n=44) | Group B (n=162) | P     |
|--------------------------------|----------------|-----------------|-------|
| Any/none                       | 18/26          | 90/72           | .091  |
| Resection                      |                |                 | .593  |
| Yes/no                         | 5/39           | 19/143          |       |
| Liver transplantation           |                |                 | 1.000 |
| Yes/no                         | 3/41           | 11/151          |       |
| RFA                            |                |                 |       |
| Yes/no                         | 3/41           | 17/145          | .577  |
| TACE                            |                |                 | .165  |
| Yes/no                         | 14/30          | 67/95           |       |
| Sorafenib, chemo-, radiotherapy|                |                 |       |
| Yes                            | 0              | 3               |       |

HCC = hepatocellular carcinoma, RFS = recurrence-free survival, TACE = transarterial chemoembolization.

Figure 2. The graph shows the OS curve of group A (liver resection plus Ta1, n=44) and group B (liver resection, n=162). Group A had better OS than group B (log-rank test, P = .014). OS = overall survival, Ta1 = thymalfasin.

Figure 3. The graph shows the RFS curve of group A (liver resection plus Ta1, n=44) and group B (liver resection, n=162). Group A had better RFS than group B (log-rank test, P = .015). RFS = recurrence-free survival, Ta1 = thymalfasin.
Table 3
Dynamic NLR changes.

| Factors | Group A (n = 43) | Group B (n = 153) | P |
|---------|-----------------|-------------------|---|
| NLR0    | 2.14 ± 0.83     | 1.91 ± 0.86       | .113 |

Group A (n = 35) Group B (n = 126)

dNLR1

Increased, % 4 (3.5) 109 (96.5) .001
Decreased, % 31 (46.4) 17 (24.6) .000

Group A (n = 34) Group B (n = 104)

dNLR3

Increased, % 4 (4.3) 90 (95.7) .001
Decreased, % 31 (68.9) 14 (31.1) .001

Group A (n = 32) Group B (n = 88)

dNLR6

Increased, % 2 (2.7) 73 (87.3) .001
Decreased, % 30 (66.7) 15 (33.3) .001

In group A, NLR0 = NLR in the 1st follow-up visit. In group B, NLR0 = NLR before administration of Ta1, dNLR1, dNLR2, dNLR3 = NLR of 2nd, 3rd, and 4th follow-up minus NLR0. NLR = neutrophil to lymphocyte ratio, Ta1 = thymalfasin.

3.5. Univariate and multivariate analysis

To identify the prognostic factors for OS and RFS of small HCC after liver resection, 17 potential variables were analyzed. As shown in Table 4, univariate analysis suggested that MVI, transfusion, total bilirubin, and Ta1 were significantly related to OS. Multivariate analysis demonstrated that MVI (P = .001, hazard ratio [HR] = 2.704, 95% confidence interval [CI] 1.510-4.842) and transfusion (P = .009, HR = 2.179, 95% CI 1.215-3.907), and Ta1 (P = .015, HR = 3.49, 95% CI 1.49-0.816) were the prognostic factors for OS of small HCC patients after liver resection. As shown in Table 5, univariate analysis suggested that MVI, tumor number, cirrhosis, HBV-DNA, and Ta1 were significantly related to the RFS. Multivariate analysis demonstrated that microvascular invasion (P = .001, HR = 2.481, 95% CI 1.615-3.812), tumor number (P = .001, HR = 2.113, 95% CI 1.380-3.234), cirrhosis (P = .023, HR = 1.966, 95% CI 1.100-3.515), and Ta1 (P = .019, HR = 5.64, 95% CI 3.49-0.910) were independent prognostic factors for RFS of small HCC patients.

Table 4
Univariate and multivariate analyses of prognostic factors for OS of 206 small HCC patients after liver resection.

| Factors                              | Univariate | Multivariate |
|--------------------------------------|------------|--------------|
|                                     | P          | HR           | 95% CI      | P          |
| Gender (F/M)                         | .658       |              |             |            |
| Age (65 vs <65 y)                    | .236       |              |             |            |
| Differentiation (H, M, L)            | .200       |              |             |            |
| MVI (yes vs no)                      | .035       | 2.704        | 1.510-4.842 | .001       |
| Tumor size, cm (3–5 vs <3)          | .300       |              |             |            |
| Tumor number (2–3 vs 1)              | .321       |              |             |            |
| Cirrhosis (yes vs no)                | .530       |              |             |            |
| HBV-naive (yes vs no)                | .061       |              |             |            |
| AFP, ng/mL (400 vs >400)             | .695       |              |             |            |
| Operation duration, min              | .683       |              |             |            |
| Transfusion (yes vs no)              | .001       | 2.179        | 1.215-3.907 | .009       |
| TBL, μmol/L                          | .031       |              | .081        |            |
| ALB, g/L                             | .398       |              |             |            |
| PT, s                                | .501       |              |             |            |
| HBV-DNA (positive vs negative)       | .255       |              |             |            |
| Preoperative antiviral therapy (yes vs no) | .446       |              |             |            |
| Thymalfasin                          | .007       | 0.349        | 0.149-0.816 | .015       |

AFP = alpha fetoprotein, ALB = albumin, CI = confidence interval, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HR = hazard ratio, MVI = microvascular invasion, OS = overall survival, PT = prothrombin time, TBL = total bilirubin.

Table 5
Univariate and multivariate analyses of prognostic factors for RFS of 206 small HCC patients after liver resection.

| Factors                              | Univariate | Multivariate |
|--------------------------------------|------------|--------------|
|                                     | P          | HR           | 95% CI      | P          |
| Gender (F/M)                         | .345       |              |             |            |
| Age (65 vs <65 y)                    | .235       |              |             |            |
| Differentiation (H, M, L)            | .761       |              |             |            |
| MVI (yes vs no)                      | .009       | 2.481        | 1.615-3.812 | <.001      |
| Tumor size, cm (3–5 vs <3)          | .907       |              |             |            |
| Tumor number (2–3 vs 1)              | .001       | 2.113        | 1.380-3.234 | .001       |
| Cirrhosis (yes vs no)                | .002       | 1.966        | 1.100-3.515 | .023       |
| HBV-naive (yes vs no)                | .233       |              |             |            |
| AFP, ng/mL (400 vs >400)             | .746       |              |             |            |
| Operation duration, min              | .302       |              |             |            |
| Transfusion (yes vs no)              | .082       |              |             |            |
| TBL, μmol/L                          | .878       |              |             |            |
| ALB, g/L                             | .360       |              |             |            |
| PT, s                                | .180       |              |             |            |
| HBV-DNA (≥10^3 vs <10^3)             | .004       | .294         |             |            |
| Preoperative antiviral therapy (yes vs no) | .055       |              |             |            |
| Thymalfasin                          | .002       | 0.564        | 0.349-0.910 | .019       |

AFP = alpha fetoprotein, ALB = albumin, CI = confidence interval, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HR = hazard ratio, MVI = microvascular invasion, PT = prothrombin time, RFS = recurrence-free survival, TBL = total bilirubin.

4. Discussion

Liver resection has been widely accepted as standard treatment for HCC patients. However, recurrence rate is high as 70% at 5 years. Risk factors for recurrence are multiple and prevalent. Consistent with previous research, our research confirmed MVI, tumor number, and cirrhosis were independent risk factors for small HCC recurrence. In small HCC, about 15% to 35% cases are with MVI,[9] about 60% to 90% cases are with cirrhosis,[10,11] and about 15% to 45% cases are multifocal.[10,12,13] Therefore, even in small HCC, 5-year RFS rate is only about 40%,[14,15] improvements in preventing recurrence and prolonging OS are essential. However, methods to prevent recurrence are limited. Adjuvant therapy, such as TACE and sorafenib, although generally used for HCC patients who have risk factors of recurrence, remains controversial.[16] TACE is restricted by invasive nature, possibility of severe complications. As for sorafenib, its application is mainly compromised by high cost which is not affordable for most HCC patients. Immunotherapy has shown some potential efficacy, but evidence is not strong enough.[17] Previous investigations have shown Ta1 may improve the outcome of unresectable HCC patients who received TACE.[7,18,19] However, little is known about its efficacy in HCC patients who received liver resection. Shuqan et al.[20] report that Ta1 plus lamivudine postoperatively compared to lamivudine...
alone may suppress HBV reaction, delay the recurrent time, and prolong the survival for HCC patients. However, their sample size was small and without stratification based on BCLC staging system. Our research is the first to focus the efficacy of Ta1 in small HCC patients who underwent liver resection. The present study demonstrated that Ta1 adjuvant therapy resulted in significant better OS and RFS than resection only. Several mechanisms may be related to the efficacy of Ta1 in improving the prognosis of HCC patients.

In recent years, accumulated evidence suggest that the systemic inflammatory response plays a significant role in various malignancies including HCC. NLR is one of the systemic inflammation markers, high postoperative NLR has been shown to be related to poor prognosis of HCC, and reduction of postoperative NLR is associated better prognosis.[15] Our research first reports that Ta1 can reduce postoperative NLR. The decline of NLR was associated with reduction of neutrophils and increment of lymphocytes. As lymphocytes have pivotal roles in inhibiting proliferation and metastatic activity of tumor cells. A relative lymphopenia may reflect deficient immune response to malignancies. On the other hand, increased neutrophils can increase the level of circulating vascular endothelial growth factor, angiopoietin-1, and matrix metalloproteinase-9 which are the factors, angiopoietin-1, and matrix metalloproteinase-9 which are involved in inhibiting proliferation and metastatic activity of tumor cells. A relative lymphopenia may reflect deficient immune response to malignancies.

Furthermore, Ta1 even suppress proliferation and induce apoptosis of tumor cells.[16] Third, postoperative active HBV replication has been proved to be associated with early HCC recurrence and shortened OS. Several studies have proved that combination of antivirus therapy and Ta1 postoperatively may be more effective in control HBV infection, and thus improve the prognosis of HCC. Therefore, Ta1 may improve the prognosis of HBV-related HCC by inhibiting HBV active replication.[20,27]

There are several limitations in our study. First, this is a retrospective analysis from our single institution, and the sample size is relatively small. Second, the analysis of the dynamic change of NLR did not incorporate all the recruited patients, because of lacking the integrity of the follow-up blood cell data and patients had clinical symptoms or signs of sepsis at the time of blood sampling for NLR. Therefore, the result of dynamic NLR change must be tested in prospective research. Third, the present study only investigates the effect of Ta1 in small HCC patients who underwent liver resection, it is uncertain its effect in other stages of HCC, or other modalities of treatment.

In conclusion, our study demonstrated that Ta1 as adjuvant therapy in small HCC after liver resection may delay recurrence and prolong OS. It is rational for small HCC patients who have high risk for recurrence after resection to receive Ta1 adjuvant therapy.

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