SHORT COMMUNICATION

Up-regulation of Human Herpesvirus 6B-derived microRNAs in the Serum of Patients with Drug-induced Hypersensitivity Syndrome/Drug Reaction with Eosinophilia and Systemic Symptoms

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Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a life-threatening multi-organ hypersensitivity reaction. Reactivation of human herpesvirus 6B (HHV-6B), which typically occurs 2–3 weeks after its onset, has been implicated in DIHS/DRESS (1). Reactivation of HHV-6 has been reported to correlate with flaring of symptoms such as fever and hepatitis (2) and renal failure (3) in patients with DIHS/DRESS, indicating that virus reactivation could contribute to some symptoms or complications in DIHS/DRESS. However, it has also been reported that reactivation of HHV-6 could be merely a result of a strong drug-specific immune response and not contribute to DRESS symptoms and severity (4).

MicroRNAs (miRNAs) play important roles in biological processes such as immune responses and cell differentiation. Herpesviruses express their own miRNAs and may regulate key viral genes (5). HHV-6A encodes miR-U86 that regulates viral lytic replication (6), while HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4 (7). However, the precise roles of these 4 miRNAs in the regulation of HHV-6B latency and reactivation remain largely unknown. Moreover, the roles of individual miRNAs in DIHS/DRESS have not yet been elucidated. The present study investigated the expression levels of the 4 HHV-6B miRNAs in the serum of patients with DIHS/DRESS during the acute and subacute stages.

MATERIALS AND METHODS (see Appendix S1[1])

RESULTS

The maximum levels of hhv6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with MPE and healthy controls ($p < 0.05$, respectively) (**Fig. 1**a).

The time course of HHV-6B miRNA expression was examined in the serum of patients with DIHS/DRESS. In case 1, HHV-6B reactivation was confirmed by detecting HHV-6B DNA in peripheral blood mononuclear cells (PBMCs) on day 25 after onset. The expression of hhv6b-miR-Ro6-2 in serum was detected on day 19, while hhv6b-miR-Ro6-4 and -1 were detected on days 25 and 33, respectively (Fig. S1a[1]).

In case 2, HHV-6B reactivation was detected on day 16 after onset. Hhv6b-miR-Ro6-2 was expressed on day 10, while hhv6b-miR-Ro6-3 and -1 were expressed on the same day as HHV-6B DNA was detected (Fig. S1b[1]).

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2925

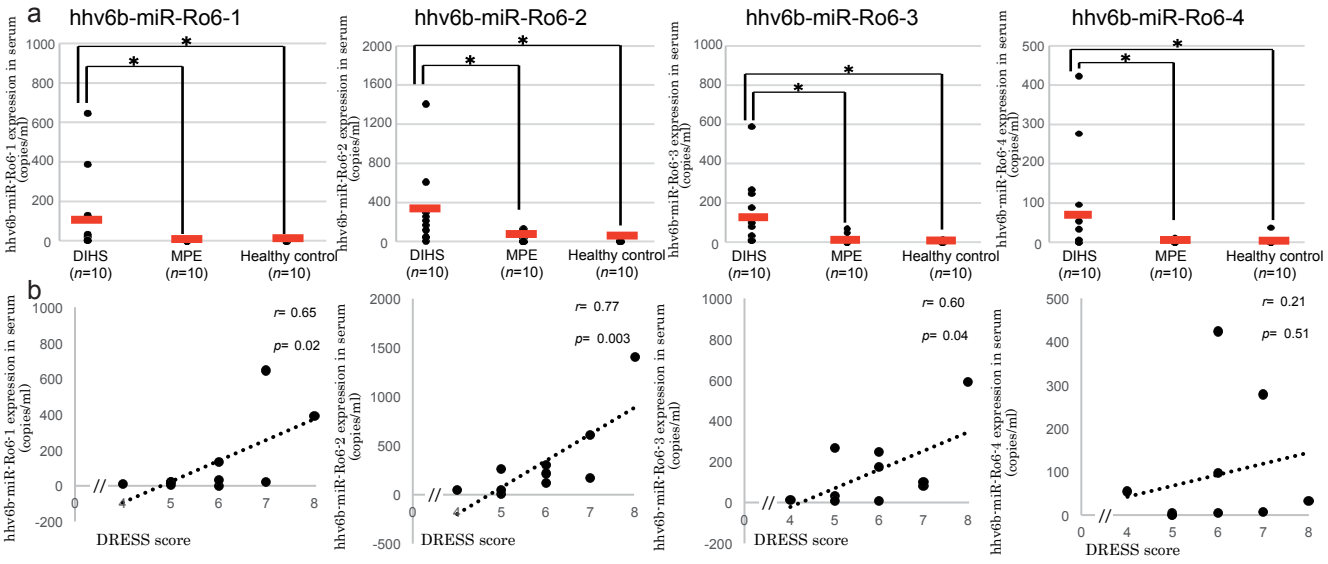

Fig. 1. (a) **Up-regulation of human herpesvirus 6B (HHV-6B)-derived miRNAs in the serum of patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS).** The maximum levels of HHV6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with maculo-papular eruption (MPE) and healthy controls. *$p < 0.05$. (b) **Correlation between DRESS scores and HHV-6B miRNAs in the serum of patients with DIHS/DRESS.** DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1, -2, and -3, respectively.

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In case 6, the expression of HHV-6B DNA and hhv6b-miR-Ro6-2 and -3 was detectable on day 9 after onset, while hhv6b-miR-Ro6-4 was detected on day 21 following hhv6b-miR-Ro6-2 expression (Fig. S1c[1]).

It was then investigated whether HHV-6B miRNA levels correlated with clinical symptoms and laboratory data. The RegiSCAR scoring system (DRESS score) was used to evaluate the severity of clinical symptoms in patients with DIHS/DRESS. Ten patients with DIHS/DRESS (4 men and 6 women) were graded according to DRESS scores as "probable" ($n=4$) or "definite" ($n=6$) (Table SI[1]). As shown in Fig. 1b, DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.65$, $p=0.02$), hhv6b-miR-Ro6-2 ($r=0.77$, $p=0.003$), and hhv6b-miR-Ro6-3 ($r=0.60$, $p=0.04$). DRESS scores were weakly associated with the serum levels of hhv6b-miR-Ro6-4 ($r=0.21$, $p=0.51$).

Relationships between the serum levels of HHV-6B miRNAs and each variable in the clinical and laboratory data were examined. The expression levels of HHV6B-derived miRNAs were not associated with liver function test results, eosinophil counts, the percentage of atypical lymphocytes, cervical lymphadenopathy, or the HHV-6B DNA levels of PBMC (data not shown). However, as shown in Fig. S2[1], the duration of fever (>38.0°C) correlated with serum levels of hhv6b-miR-Ro6-2 ($r=0.72$, $p=0.01$) and hhv6b-miR-Ro6-3 ($r=0.69$, $p=0.01$). The duration of fever was weakly associated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.30$, $p=0.34$), but not with those of hhv6b-miR-Ro6-4 ($r=0.005$, $p=0.99$).

Serum levels of hhv6b-miR-Ro6-2 were associated with the severity of skin lesions (Table SII[1]). When the expression levels of hhv6b-miR-Ro6-2 in DIHS/DRESS patients were listed in descending order, the first 8 patients with higher levels of hhv6b-miR-Ro6-2 had erythroderma, while the last 2 patients with lower levels of hhv6b-miR-Ro6-2 had diffuse MPE. hhv6b-miR-Ro6-2 may reflect the type of skin eruption. Neither Hhv6b-miR-Ro6-1, -3, nor -4 were associated with the type of skin eruption.

DISCUSSION

HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4. These 4 HHV-6B-derived miRNAs were identified in Sup-T-1 cells infected with HHV-6B using a deep sequencing approach and expressed during lytic infection (7). Hhv6b-miR-Ro6-2 and -3 are detectable very early after infection and are encoded antisense to the immediate-early (IE) genes (8). Hhv6b-miR-Ro6-1 is detected 2 days after the expression of hhv6b-miR-Ro6-2 and -3, and is encoded antisense to IE (9) or early genes (8). Hhv6b-miR-Ro6-4 is detected 4 days after HHV-6B infection (7). As shown in Fig. S1[1], our results showed that the serum levels of hhv6b-miR-Ro6-2 were increased before or at the same time as the detection of HHV-6B DNA, while those of hhv6b-miR-Ro6-1 and/or -4 were significantly increased a few weeks later than hhv6b-miR-Ro6-2 expression in some patients with DIHS/DRESS. The kinetics of the emergence of hhv6b-miR-Ro6-2, -1, and -4 in DIHS/DRESS in the present study were mostly consistent with the *in vitro* findings reported by Tuddenham et al. (7). These results suggest that hhv6b-miR-Ro6-2 and hhv6b-miR-Ro6-1/-4 have distinct functions in the regulation of HHV-6B reactivation.

We also demonstrated that the expression of hhv6b-miR-Ro6-1, -2, and -3 was associated with DRESS scores, while that of hhv6b-miR-Ro6-2 and -3 was associated with the duration of fever. These results suggest that the serum levels of HHV-6B miRNAs may be useful indicators of the severity of DIHS/DRESS.

In conclusion, the detection of the miRNAs of HHV-6B in DIHS/DRESS may reflect the reactivation of HHV-6B, and hhv6b-miR-Ro6-2 may be an early and specific biomarker for predicting the reactivation of HHV-6B. We consider these results, which were obtained by identifying a number of differentially expressed HHV-6B miRNAs in the course of DIHS/DRESS, to provide novel insights into the molecular pathogenesis of DIHS/DRESS.

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The authors have no conflicts of interest to declare.

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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

The study was reviewed and approved by the Clinical Research Ethics Committee of Nara Medical University, Nara, Japan.

The study included 10 patients with DIHS/DRESS (4 men and 6 women; median age: 63.3 years, age range 15–84 years), and 10 with maculo-papular eruption (MPE) (a milder type of drug reaction without systemic symptom) (5 men and 5 women; median age: 69.3 years, age range 25–90 years). A summary of the clinical and laboratory features of patients with DIHS/DRESS enrolled in the present study is shown in Table SI[1]. Clinical and laboratory data reported herein were obtained until 35 days after onset.

Blood samples in the acute stage were obtained from 10 patients with DIHS/DRESS at the time of their initial visits to our department. Patients with DIHS/DRESS were subjected to repeated blood sampling. Sera were separated from whole blood by centrifugation and stored at –80°C until use. Control serum samples were collected from 10 healthy volunteers.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood. DNA was isolated from PBMCs using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. In order to detect HHV-6B DNA copies, real-time PCR was performed with the Taqman Fast Advanced Master Mix (Applied Biosystems, Foster City, CA, USA), using HHV-6-specific primers and probe as described previously (10), and HHV-6B-specific primers and probe (STable I).

Isolation of miRNA from serum samples was performed with a miRNeasy Serum/Plasma Kit® according to the manufacturer's instructions with minor modifications. Complementary DNA (cDNA) was synthesized from total miRNA with a Taqman MicroRNA Reverse Transcription Kit with specific primers (miR-Ro6-1-5p, miR-Ro6-2-3p, miR-Ro6-3-3p, and miR-Ro6-4-3p) (Applied Biosystems). Quantitative real-time PCR was performed using Taqman MicroRNA Assays (miR-Ro6-1-5p, miR-Ro6-2-3p, miR-Ro6-3-3p, and miR-Ro6-4-3p) in a StepOnePlus Real Time PCR System (Applied Biosystems).

Statistical analyses were performed using a Kruskal–Wallis test. Values of $p < 0.05$ were considered significant.

STable I. Sequences of human herpesvirus 6B-specific primers and probe

Name	Sequences
Forward primer	5′-GGCTTACAGCCCCGATCAA-3′
Probe	5′-TCACAGACAAAAGAAAG-3′
Reverse primer	5′-TTCAGGAAAAAGGTTCTAACTCCAA-3′

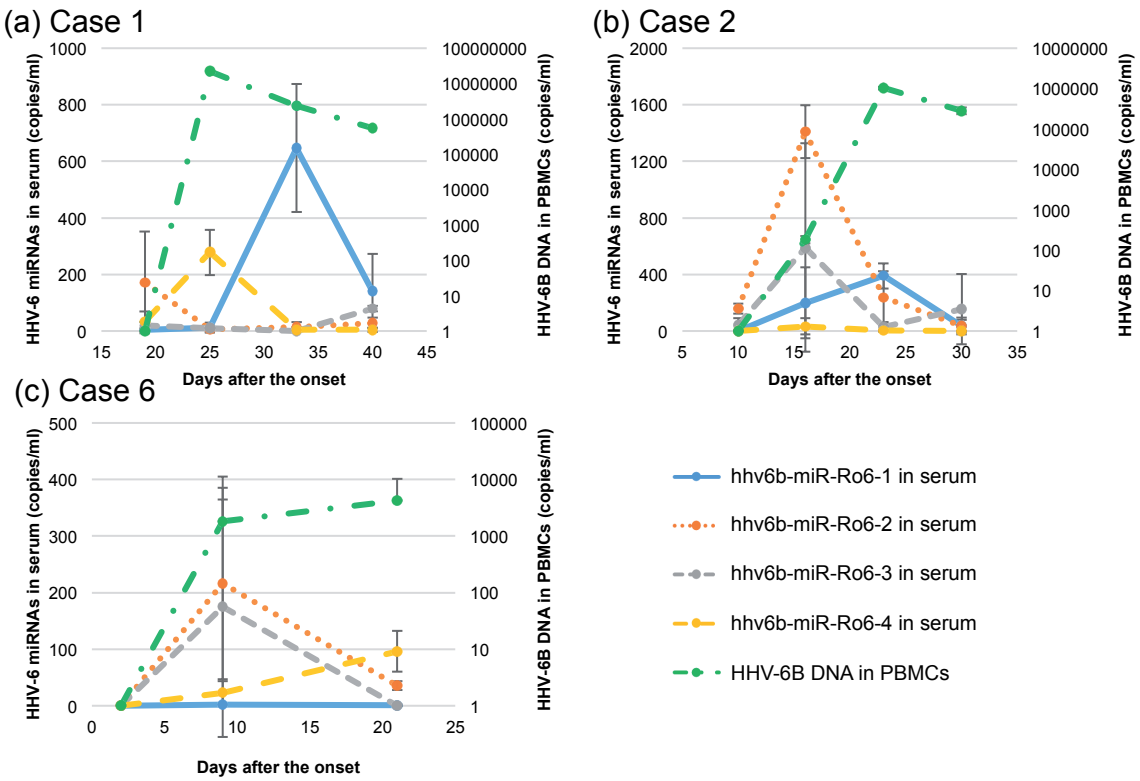

Fig. S1. The time course of expression of human herpesvirus 6B (HHV-6B) miRNAs in serum and HHV-6B DNA in peripheral blood mononuclear cells (PBMCs) from 3 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (a) Case 1. (b) Case 2. (c) Case 6.

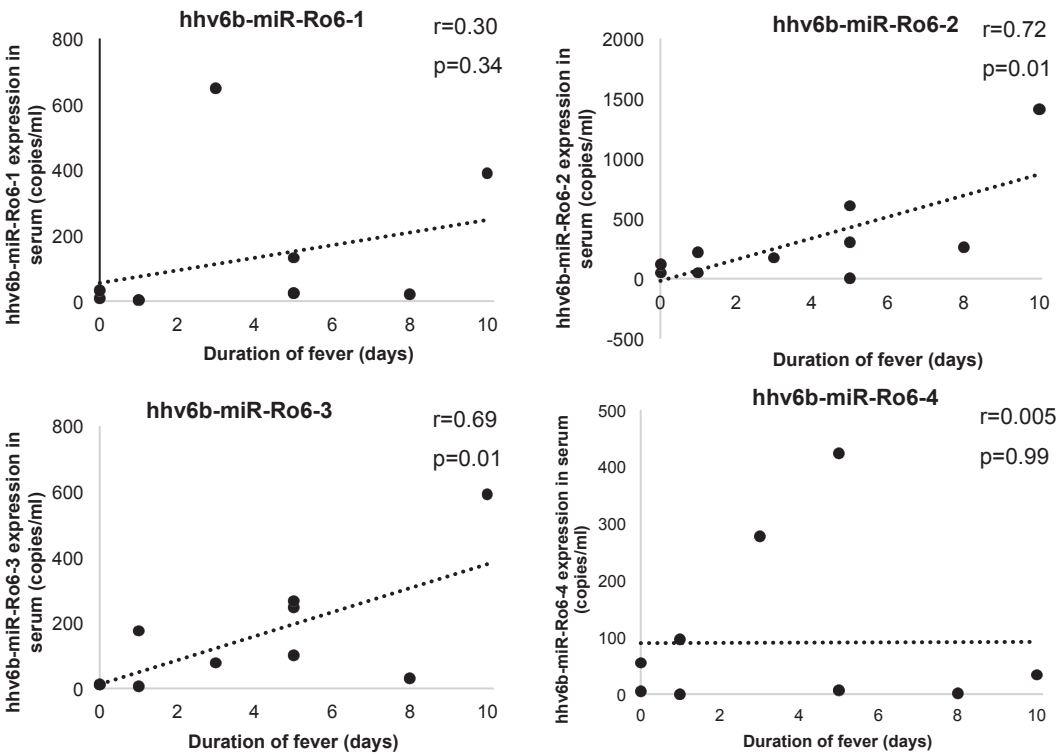

Fig. S2. Correlation between the duration of fever (>38°C) and HHV-6B miRNAs in the serum of patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). The duration of fever correlated with serum levels of hhv6b-miR-Ro6-2 and -3, respectively.

Table SI. A summary of the clinical and laboratory features of patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS)

Patient number	Sex	Age, years	Causative drug	DRESS score	Duration of fever (>38.0°C) (days)	Type of skin eruption
1	F	84	Allopurinol	7	3	Erythroderma
2	M	60	Trimethoprim-sulfamethoxazole	8	10	Erythroderma
3	F	84	Allopurinol	6	5	Erythroderma
4	F	73	Carbamazepine	4	0	Erythroderma
5	M	39	Carbamazepine	5	8	Erythroderma
6	F	15	Carbamazepine	6	1	Erythroderma
7	M	50	Modafinil	5	5	Diffuse maculo-papular eruption
8	F	77	Allopurinol	6	0	Erythroderma
9	M	68	Trimethoprim-sulfamethoxazole	5	1	Diffuse maculo-papular eruption
10	F	83	Isonicotinic acid hydrazide	7	5	Erythroderma

Table SII. Correlation between HHV-6B miRNAs and type of skin eruption

	Serum levels of HHV-6B miRNAs (copies/ml)				
Patient number	Hhv6b-miR-Ro6-1	Hhv6b-miR-Ro6-2	Hhv6b-miR-Ro6-3	Hhv6b-miR-Ro6-4	Type of skin eruption
2	389.6	1,409.3	590.3	34.0	Erythroderma
10	2.3	608.1	100.1	7.9	Erythroderma
3	131.1	297.5	246.1	423.2	Erythroderma
5	20.6	258.5	175.2	1.0	Erythroderma
6	1.9	215.9	17.5	96.3	Erythroderma
1	646.4	172.0	79.0	277.9	Erythroderma
8	31.7	122.7	10.6	4.0	Erythroderma
4	7.9	50.9	32.4	54.7	Erythroderma
9	1.1	47.0	6.2	0.1	Diffuse maculo-papular eruption
7	24.0	3.7	266.4	2.2	Diffuse maculo-papular eruption