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Case report

Quantitative evaluation of treatment response to lenalidomide by applying fluorescence *in situ* hybridization for peripheral blood granulocytes in a patient with 5q– syndrome

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The introduction of lenalidomide has significantly improved clinical outcomes in myelodysplastic syndrome (MDS) with isolated interstitial deletion of the long arm of chromosome 5 (del(5q)) (5q– syndrome). These days, MDS with isolated del(5q) includes cases with one additional chromosome abnormality other than monosomy 7 or del(7q), and so we need a better way to monitor tumor cells in each patient than the clinical parameters used to date. An 82-year-old woman with MDS with isolated del(5q) was treated with lenalidomide daily for 21 days in a 4-week cycle. Fluorescence *in situ* hybridization with *CSF1R* located at 5q was applied to the peripheral blood samples. Because mature lymphocytes are not involved in the MDS clone, based on the nuclear morphology, polymorphonuclear cells (PMNs) and round-shaped nuclear cells (RSNs) were separately evaluated during treatment. After a single course of treatment, the number of PMNs with del(5q) decreased; by the end of the second course of treatment, both PMNs and RSNs with del(5q) had disappeared. The dynamics of 5q– PMNs is a simple but rapid and reliable indicator to confirm the effect of lenalidomide in MDS with del(5q).

Keywords: fluorescence *in situ* hybridization, myelodysplastic syndrome, chromosomal abnormality, 5q– syndrome, lenalidomide

INTRODUCTION

Myelodysplastic syndrome (MDS) with isolated interstitial deletion of the long arm of chromosome 5 (del(5q)) has been established as a distinct entity since the World Health Organization (WHO) classification in 2001.[1] After its first description in 1974[2] and a review in 1985[3] by Van den Berghe *et al*., it has been referred to as 5q– syndrome because of its characteristic features: predominance in elderly women, macrocytic anemia, normal or elevated platelets and hypolobulated megakaryocytes, and medullary blast count <5% at diagnosis. The patients have an indolent clinical course and a low risk of leukemic transformation; however, most become transfusion dependent, and the subsequent iron overload may increase their risk of mortality.[4]

Remarkable hematological improvement in MDS with del(5q) has resulted from the introduction of lenalidomide, an immunomodulating agent with a wide variety of biological effects.[5-8] In a large multicenter clinical trial of lenalidomide, patients with 5q– syndrome and other MDS patients with del(5q) became transfusion independent in 67% of cases and a complete cytogenetic response was achieved in 45%.[6]

This report proposes a new method to monitor the cytogenetic response of lenalidomide using fluorescence *in situ* hybridization (FISH) applied for granulocytes in blood samples (categorized FISH).

CASE REPORT

An 82-year-old woman was referred to our hospital because of anemia. The physical examination was not remarkable, but a blood examination revealed that the white blood cell (WBC) count was 3.7 $\times 10^9$/L with no immature cells, the hemoglobin concentration was 7.6 g/dL with a mean corpuscular volume of 112.6 fL, and there was a reticulocyte count of 3.6 $\times$ 10^{10}/L and a platelet count of 14.7 $\times$

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10^{10}/L. Remarkably high concentrations were noted for erythropoietin, at 652.0 mIU/mL (4.2–23.7 mIU/mL), and ferritin at 158.0 ng/mL (3.6–114.0 ng/mL). Bone marrow (BM) aspiration from the iliac crest showed mildly hypocellular BM with 3.0% myeloblasts, erythropoietic hypoplasia, and an increased number of megakaryocytes with small nonlobated or hypolobated nuclei (Figure 1), which was consistent with the BM biopsy, which showed mild hypocellularity

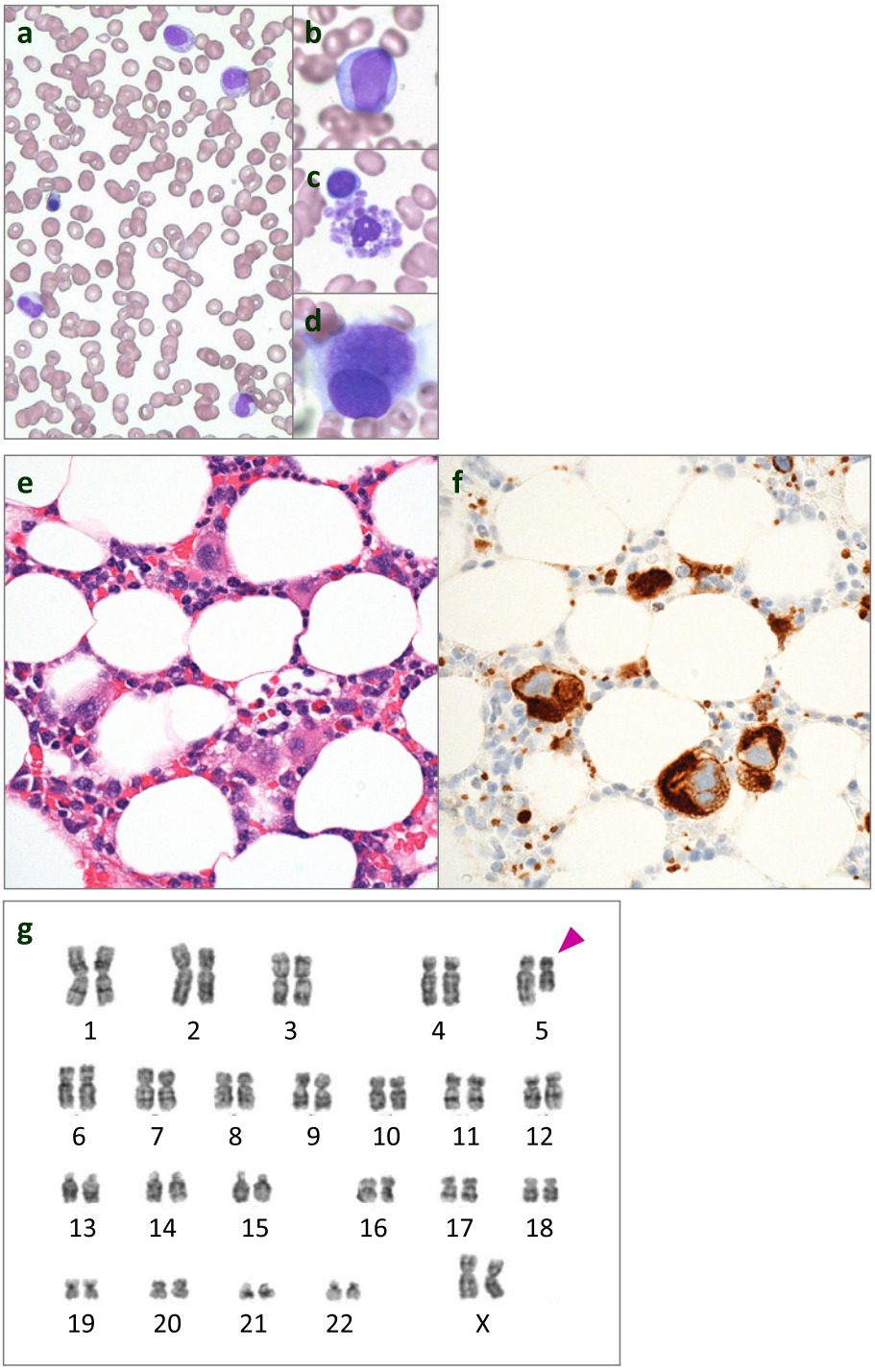

Fig. 1. Bone marrow. (***A–D***) May–Grünwald–Giemsa staining of the aspirated bone marrow (BM) smear. BM is slightly hypocellular (***A***) containing myeloblasts (***B***), micromegakaryocytes (***C***), and megakaryocytes with small nonlobated or hypolobated nuclei (***D***) (original magnification, 400× (***A***) and 1000× (***B–D***)). (***E***, ***F***) Histologic findings of the BM biopsy specimen (original magnification, 400×). BM is hypoplastic (hematoxylin and eosin staining (***E***)) with an increased number of megakaryocytes (immunohistochemical staining with anti-CD42b antibodies (***F***)). (***G***) A representative G-banded karyotype of 46,XX,del(5)(q15q33). The arrowhead indicates the chromosome with an interstitial deletion of the long arm of chromosome 5.

with an increase in megakaryocytes (Figure 1). Chromosome analysis of the aspirated BM showed 46,XX,del(5)(q15q33)[12]/46,XX[8] (Figure 1).

The patient was diagnosed with MDS with isolated del(5q) as defined by the WHO classification,[9] and classified as intermediate risk according to the Revised International Prognostic Scoring System.[10] Treatment was initiated with 10 mg lenalidomide daily for 21 days in a 4-week cycle (Figure 2). During the first course of treatment, the WBC and platelet counts decreased to 3.0×10^9/L and 3.2×10^{10}/L, respectively. Because the hemoglobin concentration also decreased to 5.6 g/dL, the patient received 2 units of red blood cell transfusion once. Her erythropoietin level increased to 1030.0 mIU/mL after the first treatment, and then gradually decreased following a peak value of 1060.0 mIU/mL. From the second course of treatment, the dose of lenalidomide had to be reduced to 5 mg owing to generalized pruritus, but the patient's hemoglobin concentrations began to increase steadily and were normalized by the end of the sixth course of treatment. While the mean corpuscular volume and ferritin concentration decreased, the reticulocyte count remained within the normal range. Furthermore, the WBC and platelet counts returned to a normal range by the end of the third course of treatment, during which time the lymphocyte ratio was maintained at a high level of up to 55.5%. Bone marrow aspiration was proposed to confirm remission, but the patient did not agree to it because of a fear of pain.

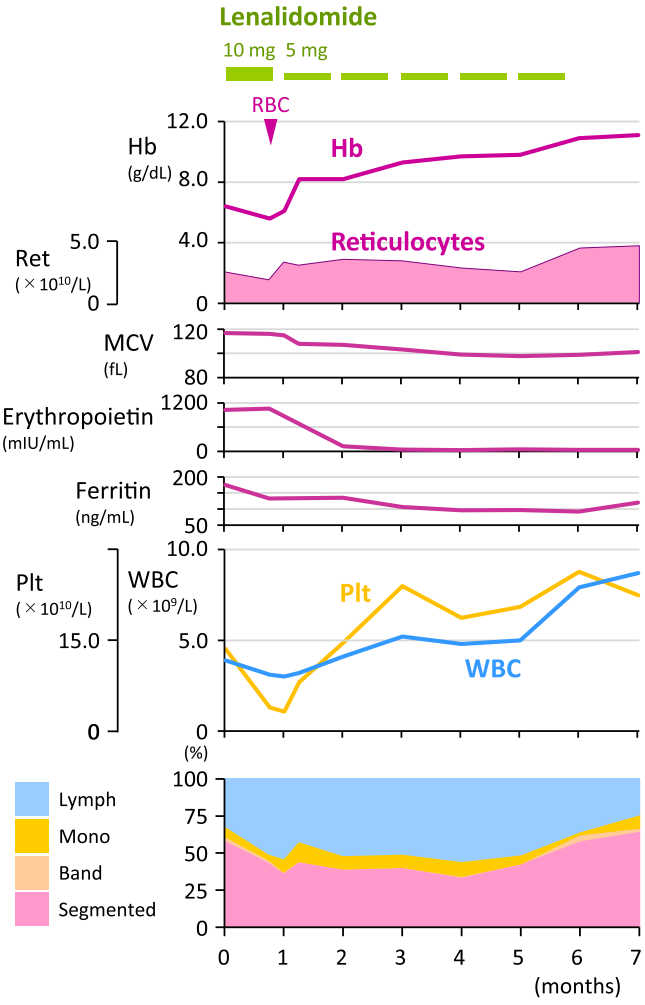

Fig. 2. Clinical course.
RBC, red blood cell transfusion; Hb, hemoglobin concentration; Ret, reticulocytes; MCV, mean corpuscular volume; WBC, white blood cell count; Plt, platelet count; Lymph, lymphocyte; Mono, monocyte; Band, band cell; Segment, segmented cell.

CATEGORIZED FISH

Methods

The method of categorized FISH has been described in detail elsewhere.[11,12] Briefly, each peripheral blood (PB) sample was subjected to hypotonic treatment with 0.075 M KCl for 15 minutes and fixation with methanol and acetic acid (3:1). Next, FISH for *CSF1R* located at 5q was performed using Vysis LSI CSF1R SpectumOrange/D5S23, D5S721 SpectrumGreen probes (Abbot Molecular, IL, USA) according to the manufacturer's instructions. We tried to exclude lymphocytes for the evaluation of therapeutic effects because, when investigated by FISH, mature lymphocytes in 5q– syndrome are generally not involved in the MDS clone.[9,13] Based on the nuclear morphology, we separately observed a total of 100 each of polymorphonuclear cells (PMNs) and round-shaped nuclear cells (RSNs).

Results

Since the category of RNSs comprised a mixture of band cells, monocytes, and lymphocytes in the patient, the PB leukocytes were categorized into the following 4 cell populations using FISH (Figure 3, Figure 4):

(1) RSNs with defect of *CSF1R* (5q– RSNs): mostly neoplastic monocytes and few band cells.
(2) PMNs with defect of *CSF1R* (5q– PMNs): neoplastic segmented cells.
(3) RSNs without defect of *CSF1R* (normal RSNs): normal cells containing monocytes, lymphocytes, and few band cells.
(4) PMNs without defect of *CSF1R* (normal PMMs): normal segmented cells.

The number of 5q– PMNs, which accounted for 46% at presentation, gradually decreased and disappeared by the end of the second course of treatment (Figure 5). In contrast, the 5q– RSNs maintained their number after the first course of treatment but then also disappeared after the second course of treatment. The normal PMNs made a relatively slow recovery and increased significantly at around the same time as the recovery from anemia. The increase of lymphocytes was reflected in the high value of normal RSNs.

DISCUSSION

Lenalidomide is highly effective for MDS with del(5q) not only hematologically but also cytogenetically,[6] and so a simple method is needed to evaluate its cytogenetic response

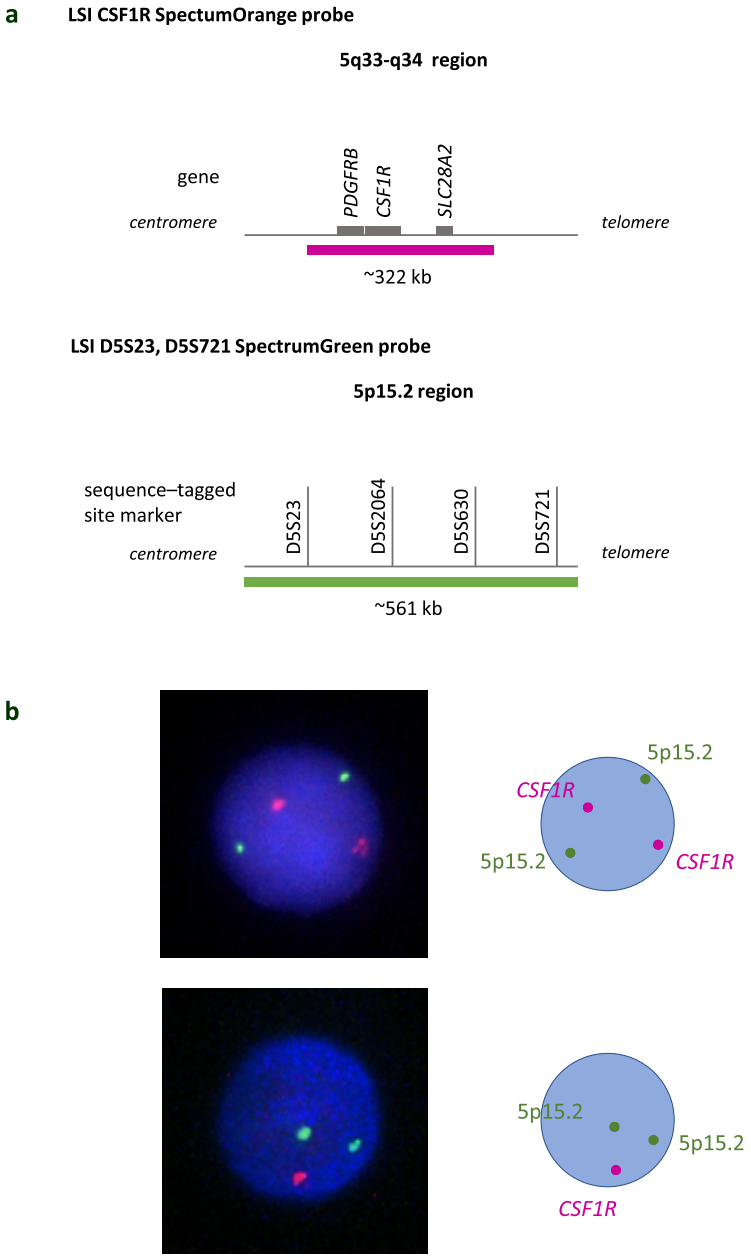

Fig. 3. Representative results of FISH for *CSF1R*. (***A***) Schematic views of the genomic locations of the probes (Vysis LSI CSF1R SpectumOrange/ D5S23, D5S721 SpectrumGreen probes). (***B***) Representative results and schematic presentations of fluorescence *in situ* hybridization. Two signals each for *CSF1R* and *5p15.2* are detected in a normal cell hybridized with these probes (upper), whereas 1 signal for *CSF1R* and 2 signals for *5p15.2* in tumor cells are detected (lower).
FISH, fluorescence *in situ* hybridization.

in the early phase of the disease. Conventional karyotypic analysis is essential for the diagnosis of MDS with del(5q), but this analysis has a limitation in that the target cells are restricted to dividing cells; therefore, BM analysis is necessary, and its aspiration is inevitable for the evaluation. The aspiration of BM is an invasive procedure for patients and sometimes fails to acquire an adequate specimen for its correct assessment. In contrast, once an appropriate genetic marker is discovered by chromosome analysis, FISH gains the advantage of being applicable to non-dividing mature cells of PB, which is preferable to BM for frequent sampling. However, the appropriate quantitative evaluation of the therapeutic effect using PB is interfered with by the fraction of mature lymphocytes, which are not involved in the del(5q) clone, except for in very rare cases.[14,15] For a solution to this problem, the FISH results were categorized based on the nuclear morphology into segmented cells (PMNs) derived from the stem cells of MDS and other cells (RSNs) contain-

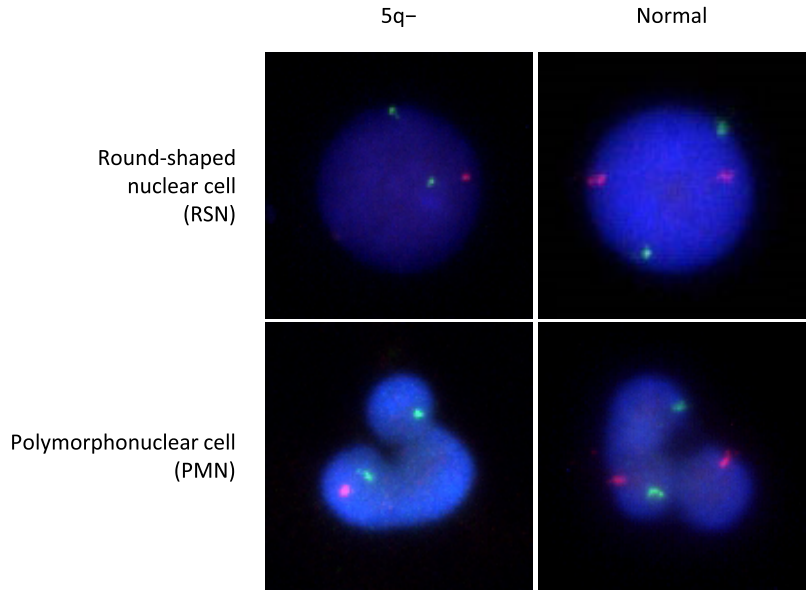

Fig. 4. Representative results of categorized FISH. RSN and PMN with defect of *CSF1R* (left upper and lower, respectively) and RSN and PMN without defect of *CSF1R* (right upper and lower, respectively).
PMN, polymorphonuclear cell; RSN, round-shaped nuclear cell.

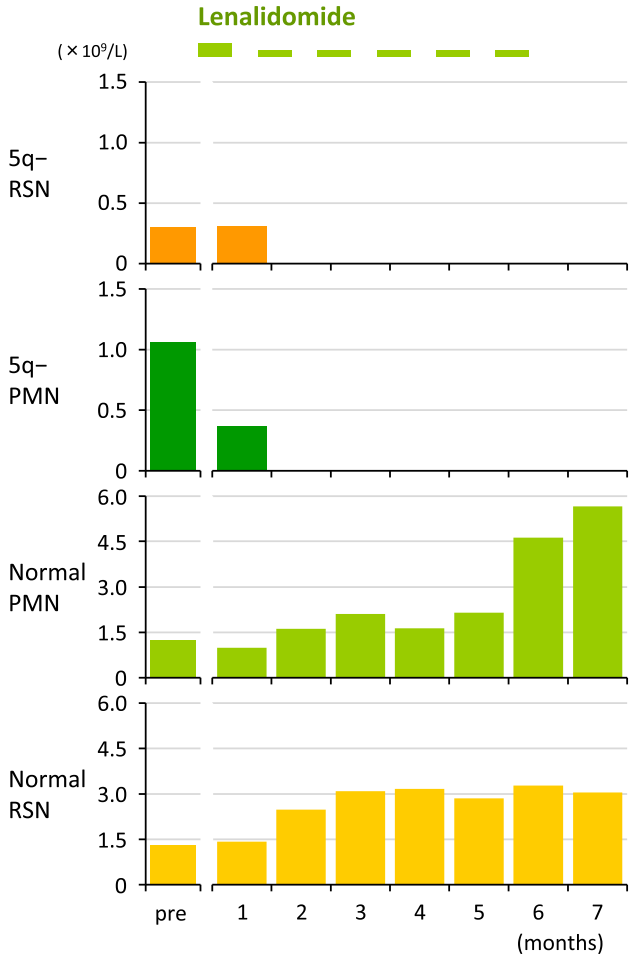

Fig. 5. Cell kinetics by the categorized FISH and WBC counts with differentials.
WBC, white blood cell; Lymph, lymphocyte; Mono, monocyte; Band, band cell; Segmented, segmented granulocyte.

ing mature lymphocytes of normal lineage. The therapeutic effect of lenalidomide was successfully monitored by categorized FISH until the complete cytogenetic and hematological response.

In the revised 4th edition of the WHO classification, MDS with isolated del(5q) is now allowed to include cases with one additional chromosome abnormality other than monosomy 7 or del(7q).[9] Because the therapeutic effect of lenalidomide may be variously affected by each abnormality in addition to del(5q), each patient needs a better way to evaluate its effectiveness. The ratio of 5q– PMNs after a single course of treatment with lenalidomide was the earliest indicator among the clinical parameters in this case, such that the categorized FISH was expected to have the potential to predict the treatment outcome of del(5q) not only in isolation, but also with other cytogenetic abnormalities. The reappearance of 5q– PMNs can be also used as the earliest sign of relapse, because >50% of segmented cells had already been replaced by the del(5q) clone at presentation when the WBC was normal and did not show any dysplastic changes.

To date, we have analyzed the pattern of tumor cell kinetics by categorized FISH in chronic myeloid leukemia (CML) treated with the tyrosine kinase inhibitor (TKI)[16] and acute promyelocytic leukemia by the all-*trans* retinoic acid.[11,12] Because the kinetic pattern of the current case was similar to that for the *BCR-ABL*-positive PMNs of CML treated with the TKI, the antitumor effect of lenalidomide was considered to suppress the growth and facilitate the apoptosis of tumor cells. While the number of 5q– PMNs gradually decreased and disappeared, the 5q– RSNs maintained their number and then disappeared. Because immature granulocytes were not observed during the observation period, the difference between their kinetic patterns may be derived from the lifes-

pans of granulocytes and monocytes.

Although further analyses of cell kinetics are needed, categorized FISH can be a simple but rapid and reliable method to confirm the cytogenetic response of lenalidomide in MDS with del(5q).

CONFLICT OF INTEREST

A. Arai reports grants from Ono Pharmaceutical and Kyowa Kirin outside the submitted work. The other authors have nothing to disclose.

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