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MYC amplification on double minute chromosomes in plasma cell leukemia with double *IGH/CCND1* fusion genes

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ABSTRACT

In multiple myeloma (MM), *MYC* rearrangements that result in increased *MYC* expression are associated with an aggressive form of MM and adverse outcome. However, the consequences of *MYC* amplification in MM remain unclear. Here, we describe an unusual case of plasma cell leukemia (PCL) harboring *MYC* amplification on double minute chromosomes (dmin). A 79-year-old woman was initially diagnosed as having BJP-κ type MM with bone lesions. After seven months, the disease progressed to secondary PCL: leukocytes 49.1×10^9/L with 77% plasma cells showing lymphoplasmacytic appearance. The bone marrow was infiltrated with 76% plasma cells immunophenotypically positive for CD38 and negative for CD45, CD19, CD20, and CD56. The karyotype by G-banding and spectral karyotyping was 48,XX,der(14)t(11;14)(q13;q32),+der(14)t(14;19)(q32;q13.1),+18,6~95dmin[15]/46,XX[5]. Fluorescence *in situ* hybridization detected multiple *MYC* signals on dmin and double *IGH/CCND1* fusion signals on der(14)t(11;14) and der(14)t(14;19). Most plasma cells were diffusely and strongly positive for MYC and CCND1 by immunohistochemistry. The patient died of progressive disease after one week. *MYC* amplification led to high expression of MYC and rapid disease progression, indicating its clinical significance in the pathogenesis of MM/PCL. *MYC* amplification on dmin may be a very rare genetic event closely associated with the progression to PCL and coexistence of *IGH/CCND1* fusions.

Key words**:** *MYC* amplification, double minute chromosomes, multiple myeloma, plasma cell leukemia, *IGH/CCND1* fusion gene

Introduction

Multiple myeloma (MM) is a B-cell malignancy characterized by proliferation of monoclonal plasma cells in the bone marrow; the disease infrequently progresses to plasma cell leukemia (PCL), which is defined by the presence of circulating plasma cells in the peripheral blood [1, 2]. Cytogenetically, MM is primarily divided into a hyperdiploid group harboring numerous trisomies, and a non-hyperdiploid group in which the majority of cells have recurrent *IGH* translocations such as t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23). On the other hand, *MYC* abnormalities as well as del(17p13) and -13/del(13q) are considered as secondary genetic events associated with disease progression [1]. The *MYC* gene located at 8q24 encodes a transcription factor that regulates cell proliferation, growth, metabolism and apoptosis [3]. *MYC* is activated mainly by translocations or less commonly by numerical changes including amplification, and *MYC* translocations are observed in about 15% of patients with MM [3, 4]. *MYC* abnormalities that result in increased *MYC* expression are associated with an aggressive form of MM, high incidence of PCL and extramedullary disease, and an adverse outcome [3-6]. These findings suggest that *MYC* dysregulation is one of the major molecular events in the pathogenesis of MM/PCL [5, 6]. However, only a few cases of MM/PCL with *MYC* amplification have been described [6-9].

Double minute chromosomes (dmin) that are small, acentric, usually spherical chromatin bodies represent a form of extrachromosomal gene amplification and reflect disease progression in human tumors. In hematological malignancies, dmin are observed in about 1% of patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [10, 11]. The most commonly amplified gene in AML with dmin is *MYC*, and less commonly *KMT2A*. In comparison, MM with dmin and its association with *MYC* have hardly been analyzed [6]. Here, we describe an unusual case of MM that evolved to secondary PCL harbor-

ing *MYC* amplification on dmin and double *IGH/CCND1* fusions.

Materials and methods

Case history

A 79-year-old woman was admitted to another hospital because of back pain. Magnetic resonance imaging showed bone destruction of Th5, Th7, and Th8. Bone biopsy led to a diagnosis of plasma cell neoplasm, and the patient was referred to our hospital for further evaluation. Peripheral blood values were hemoglobin 13.4 g/dL, platelets 341 × 10^9/L and leukocytes 3.8 × 10^9/L with no plasma cells. Serum protein electrophoresis did not show a monoclonal band, whereas urine protein electrophoresis and immunofixation detected κ-type Bence-Jones protein (BJP). The bone marrow was slightly hypocellular with 52.2% myeloid cells, 4.0% monocytes, 7.4% lymphocytes, 21.2% erythroblasts, and 12.0% plasma cells (Fig. 1A). Bone marrow biopsy also showed slightly hypocellular marrow with slight increase of plasma cells that were positive for CD138 (Fig. 2AB). Radiographic bone survey disclosed osteolytic lesions in the left femur. We made a diagnosis of symptomatic MM, BJP-κ type. The patient received bortezomib and dexamethasone (Bd) therapy and monthly infusion of zoledronic acid, but a pathological fracture of the left femur occurred. After operation, radiation therapy for the left femur and Th5-Th7 and four cycles of Bd therapy were added.

Seven months after diagnosis, she was admitted to our hospital because of an increased number of plasma cells in the peripheral blood: hemoglobin 9.6 g/dL, platelets 117 × 10^9/L and leukocytes 49.1 × 10^9/L with 1% metamyelocytes, 2% band forms, 15% segmented neutrophils, 1% eosinophils, 3% monocytes, 1% lymphocytes, and 77% plasma cells that presented lymphoplasmacytic morphology (Fig. 1B). The bone marrow was hypercellular with 76.0% plasma cells, 12.2% myeloid cells, 5.6% lymphocytes, and 2.8% erythroblasts. Im-

munophenotyping revealed that the CD45-negative gated cells were positive for CD38 and CD33 but negative for CD19, CD20, and CD56 (Fig. 1C). Bone marrow biopsy showed hypercellular marrow with diffuse proliferation of plasma cells that were strongly positive for CD138 but negative for CD20 and CD56 by immunohistochemistry (Fig. 2EF). A diagnosis of transformation to secondary PCL was made. The patient was treated with carfilzomib and dexamethasone without apparent effect. After one week, leukocytes increased to 135.7 × 10^9/L with 88% plasma cells, and she died of progressive disease.

Chromosome analyses and spectral karyotyping (SKY)

Chromosome analyses were performed using the G-banding technique on short-term cultured bone marrow cells. Karyotypes were described according to ISCN 2016. SKY analysis was carried out with Sky Paint Probes (Applied Spectral Imaging, Carlsbad, CA, USA) on five metaphase spreads. Chromosomes were counterstained with 4', 6-diaminido-2-phenylindole dihydrochloride (DAPI).

Fluorescence in situ hybridization (FISH) analyses

We used Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probe Kit and Vysis LSI IGH/CCND1 Dual Fusion Probe Kit (Abbott Molecular, Abbott Park, IL, USA). FISH analyses were performed on 20 metaphase spreads.

Results

Chromosome analyses of bone marrow cells at diagnoses of MM and PCL showed 47,XX,+mar[1]/46,XX[19] and 48,XX,+add(14)(q32),der(14)t(11;14)(q13;q32),+18,6~95dmin[15]/46,XX[5], respectively (Fig. 3AB). SKY confirmed der(14)t(11;14)(q13;q32) and revealed that add(14)(q32) was

der(14)t(14;19)(q32;q13.1). However, the origin of the dmin could not be identified because its color did not correspond to any of the colors displayed on normal chromosomes (Fig. 3C). The colors of dmin seemed to be similar although it was difficult to distinguish the colors definitely due to the small sizes of dmin. To analyze the possible association between *MYC* and dmin, we performed FISH with an *IGH/MYC*/CEP 8 probe and detected multiple *MYC* signals on dmin in 15 of 20 metaphase spreads (Fig. 3D). Unexpectedly, two *MYC* signals remained on two normal chromosomes 8. Due to the coexistence of t(11;14)(q13;q32), we next performed FISH with an *IGH/CCND1* probe, and detected *IGH/CCND1* fusion signals on der(14)t(11;14) and der(14)t(14;19) in 15 of 20 metaphase spreads (Fig. 3E). Furthermore, immunohistochemistry confirmed that most plasma cells were diffusely and strongly positive for CCND1 and MYC (Fig. 2GH). We also retrospectively performed immunohistochemistry on bone marrow biopsy at initial diagnosis of MM. Plasma cells were positive for CCND1 but almost negative for MYC (Fig. 2CD).

Discussion

We have detected *MYC* amplification in the form of dmin in secondary PCL with duplicated *IGH/CCND1* fusions. These genetic aberrations led to high expression of MYC and CCND1, rapid disease progression, and short survival, indicating their clinical significance in the pathogenesis of MM/PCL. Immunohistochemistry confirmed that CCND1 was already positive at initial diagnosis of MM whereas MYC became positive at diagnosis of PCL. Thus, CCND1 overexpression was thought to be an early pathologic event, and *MYC* amplification leading to MYC overexpression may be a secondary genetic change relating to the disease progression.

MYC amplification in MM was initially studied by Southern blot analysis. An 8- to

32-fold amplification was found in 2 of 3 PCL cases but none of 21 MM cases, suggesting that *MYC* amplification could be related to the more malignant phenotype [7]. Two PCL cases presented higher level of *MYC* expression and hyperleukocytosis (122×10^9/L and 187×10^9/L), as observed in the present case. Again, using Southern blot, the second study showed 5 to 10 times *MYC* amplification in 2 of 26 MM cases [8]. The third study used interphase-FISH and found *MYC* amplification with >10 signals in 2 of 39 MM cases; one had an *IGH/CCND1* fusion and the other had a leukemic change [9]. Furthermore, PCR analysis demonstrated that *MYC* amplifications were uncommon in primary myeloma cells although they were found in 6 of 11 myeloma cell lines (maximum 9 copies) [12]. However, cytogenetic mechanisms leading to *MYC* amplification were unclear in these studies.

Dmin seem to be an unusual cytogenetic aberration in MM. In the Mitelman database, among 2,246 cases of plasma cell neoplasms with cytogenetic abnormalities, only 19 cases (0.85%, 18 MM and 1 PCL) had dmin [13]. These cases were merely described without detailed data in the literature, and only one case was shown to be associated with *MYC* amplification [6]. This case was a 52-year-old woman with primary PCL. The karyotype was 46,XX,der(7)t(X;7)(q23;p21),der(14)t(11;14)(q13;q32),add(19)(q13),dmin. Metaphase-FISH showed a variable degree of *MYC* amplification on dmin in only ~10% of cells in the malignant clone. In contrast, we first demonstrated the image of *MYC* amplification on dmin. The amplification level was very high (>60 times), and dmin were detected in all malignant cells, indicating the pivotal role of highly amplified *MYC* in the pathogenesis of MM/PCL. Our results, together with previous reports, suggest that *MYC* amplification on dmin may be a very rare genetic event closely associated with the progression to PCL and coexistence of *IGH/CCND1* fusions.

MYC amplification is frequently accompanied by amplification of the *PVT1* gene, which locates downstream of *MYC*, encodes a long non-coding RNA, and plays an important role

in tumorigenesis [14]. Similarly, in the present case, there is a possibility that other genes including *PVT1* located near *MYC* at 8q24 were co-amplified. In addition, we found two remarkable cytogenetic events. First, SKY revealed that the color of dmin did not correspond to that of chromosome 8 although amplification of *MYC* at 8q24 occurred on dmin. In short, SKY was not able to identify the origin of dmin. In contrast, we previously demonstrated that dmin and ring chromosomes with *MYC* amplification in AML were completely labeled with the color of chromosome 8 by SKY [11, 15]. Thus, it is possible that dmin contained chromosomal segments other than chromosome 8, suggesting that additional oncogenes originating from other chromosomes might be co-amplified with *MYC*. It is also possible that chimeric genes containing genes located at 8q24 and other oncogenes were formed on dmin [14]. Second, a *MYC* signal was not deleted from one of the two normal copies of chromosomes 8, and both *MYC* signals remained. A cryptic *MYC* deletion in one chromosome 8 homolog has been shown in the majority of MDS/AML with *MYC*-containing dmin [10]. Here, DNA segments including *MYC* are excised from chromosome 8, circularized, and amplified to produce dmin. This “episome model” is unlikely to explain the mechanism to amplification in the present case. Accordingly, it is possible that the molecular mechanism leading to *MYC* amplification on dmin is different between AML and MM/PCL.

We confirmed that *MYC* amplification led to MYC protein overexpression in most bone marrow plasma cells. Recently, Møller et al. demonstrated that MYC protein was expressed in 92% of MM cases by immunohistochemistry: MYC-positivity ranged from 1% to 65% of CD138-positive plasma cells [16]. Overall survival was significantly inferior if MYC expression was found in >40% of plasma cells. In the present case, at least 80% of plasma cells were positive for MYC at diagnosis of PCL. It is possible that this marked high MYC-positivity induced high proliferative capacity and an adverse prognosis. Furthermore, a positive correlation between MYC expression and sensitivity to the MYC activity inhibitor

has been presented in myeloma cell lines [12]. Thus, patients with myeloma cells expressing high MYC levels might benefit from MYC inhibition.

The t(11;14)(q13;q32) translocation, which is observed in about 15% of MM and 20–40% of PCL, results in an *IGH/CCND1* fusion gene on der(14)t(11;14) and CCND1 overexpression [1, 2, 17]. In the present case, *IGH/CCND1* fusion signals were detected on der(14)t(14;19)(q32;q13.1) as well as der(14)t(11;14)(q13;q32), suggesting that interstitial insertion of 11q including *CCND1* occurred between 19q13.1 and *IGH* at 14q32. Similarly, duplication of the *IGH/CCND1* fusion signal was found on der(14)t(11;14) in three of four primary PCL cases [6]. Thus, duplication of *IGH/CCND1* may be a recurrent genetic event in PCL. Morphologically, lymphoplasmacytic appearance, which was associated with t(11;14)(q13;q32), was also observed in the peripheral blood [17]. It will be important to clarify the functional association between *MYC* amplification and duplicated *IGH/CCND1* fusions in the pathogenesis of MM/PCL.

Conflict of interest

Dr. Minami reports honoraria from Janssen, Novartis, and Ono, and research grant from Ono. Other authors have no conflict of interest.

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Figure legends

Fig. 1.

(A) Bone marrow smears at initial diagnosis of MM show plasma cells with mature morphology (May-Grünwald-Giemsa staining, ×1000).

(B) Peripheral blood smear at diagnosis of PCL shows small to medium-sized plasma cells with high nuclear/cytoplasmic ratio and lymphoplasmacytic morphology (May-Grünwald-Giemsa staining, ×1000).

(C) Flow cytometric findings of bone marrow cells at diagnosis of PCL by CD45/side scatter (SSC) gating. The corresponding cell percentage demarcated by the gate is 80.6%. The results of two-color analyses with CD38 and CD103, CD19 and CD13, CD11c and CD20, CD33 and CD2, CD56 and CD16, and λ-chain and κ-chain for gated cells are shown. Corresponding cell percentages in each fraction are indicated. The CD45-negative gated cells are positive (>20%) for CD38 (88.6% of the gated cells) and weakly positive for CD33 (26.3%), but negative for CD19, CD20, CD56, and surface κ and λ chains.

Fig. 2. Pathological findings of the bone marrow at initial diagnosis of MM (A to D) and at diagnosis of PCL (E to H).

(A) H&E-stained bone marrow biopsy shows slightly hypocellular marrow with slight increase of atypical plasma cells (×400).

(B) -(D) Immunohistochemistry of bone marrow biopsy (×400). A cluster of plasma cells is positive for CD138 (B) and CCND1 (C) but almost negative for MYC (D).

(E) H&E-stained bone marrow biopsy shows diffuse proliferation of atypical plasma cells (×200).

(F)-(H) Immunohistochemistry of bone marrow biopsy (×200). Almost all cells are diffusely and strongly positive for CD138 (F). Most plasma cells are also diffusely and strongly positive for CCND1 (G) and MYC (H).

Fig. 3.

(A) G-banded karyotype of bone marrow cells at diagnosis of PCL: 48,XX,+add(14)(q32),der(14)t(11;14)(q13;q32),+18,6~95dmin. The left and right arrows indicate add(14)(q32) and der(14)t(11;14), respectively.

(B) A metaphase cell demonstrates multiple extrachromosomal dmin.

(C) Spectral karyotyping of metaphase spreads after spectrum-based classification (left side, reverse DAPI; right side, SKY). The karyotype is revised as 48,XX,der(14)t(11;14)(q13;q32),der(14)t(14;19)(q32;q13.1),+18,dmin. Arrows indicate rearranged chromosomes. The color of dmin does not correspond to any of the colors found on normal chromosomes.

(D) FISH with Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probe Kit on metaphase spreads and interphase nuclei. Multiple (>10) *MYC* (red) signals are detected on multiple dmin in 15 of 20 metaphase spreads. In addition, two *MYC* signals remain on two normal chromosomes 8. Arrows indicate 1) *MYC* (red) and CEP 8 (blue) signals on normal chromosomes 8, 2) an *IGH* (green) signal on a normal chromosome 14, 3) a split *IGH* signal on der(14)t(11;14)(q13;q32), and 4) a split *IGH* signal on der(14)t(14;19)(q32;q13.1). Two blue, three green, and multiple red signals are also observed on an interphase nucleus (inset).

(E) FISH with Vysis LSI IGH/CCND1 Dual Fusion Probe Kit on metaphase spreads and interphase nuclei. Two *IGH/CCND1* fusion signals are detected in 15 of 20 metaphase spreads. Arrows indicate 1) *CCND1* (red) signals on normal chromosomes 11, 2) an *IGH*

(green) signal on a normal chromosome 14, 3) an *IGH/CCND1* (red/green, yellow) fusion signal on der(14)t(11;14)(q13;q32), and 4) an *IGH/CCND1* fusion signal on der(14)t(14;19)(q32;q13.1). Two red, one green, and two yellow signals are also observed on an interphase nucleus (inset).

Fig. 1

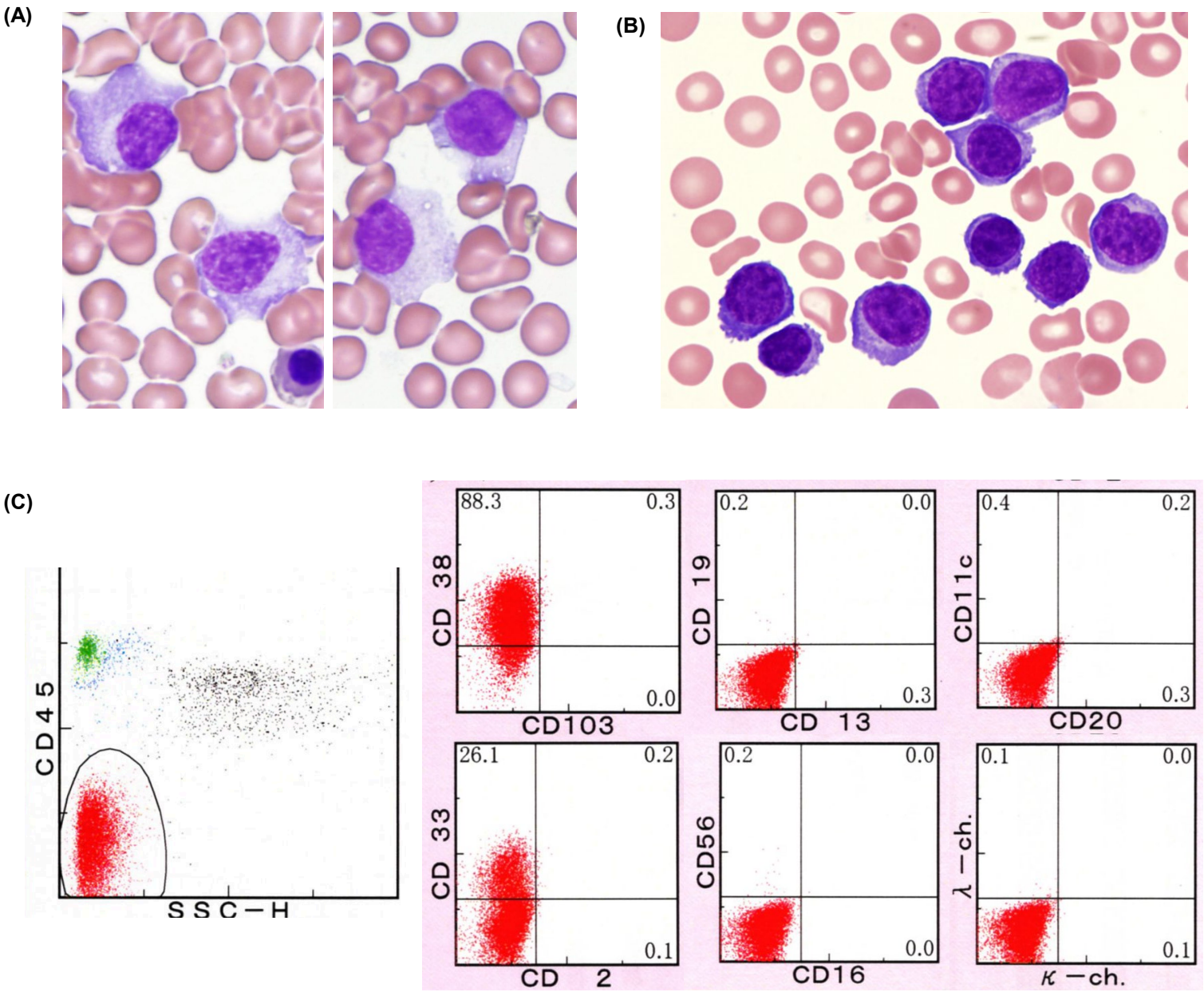
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Fig. 2

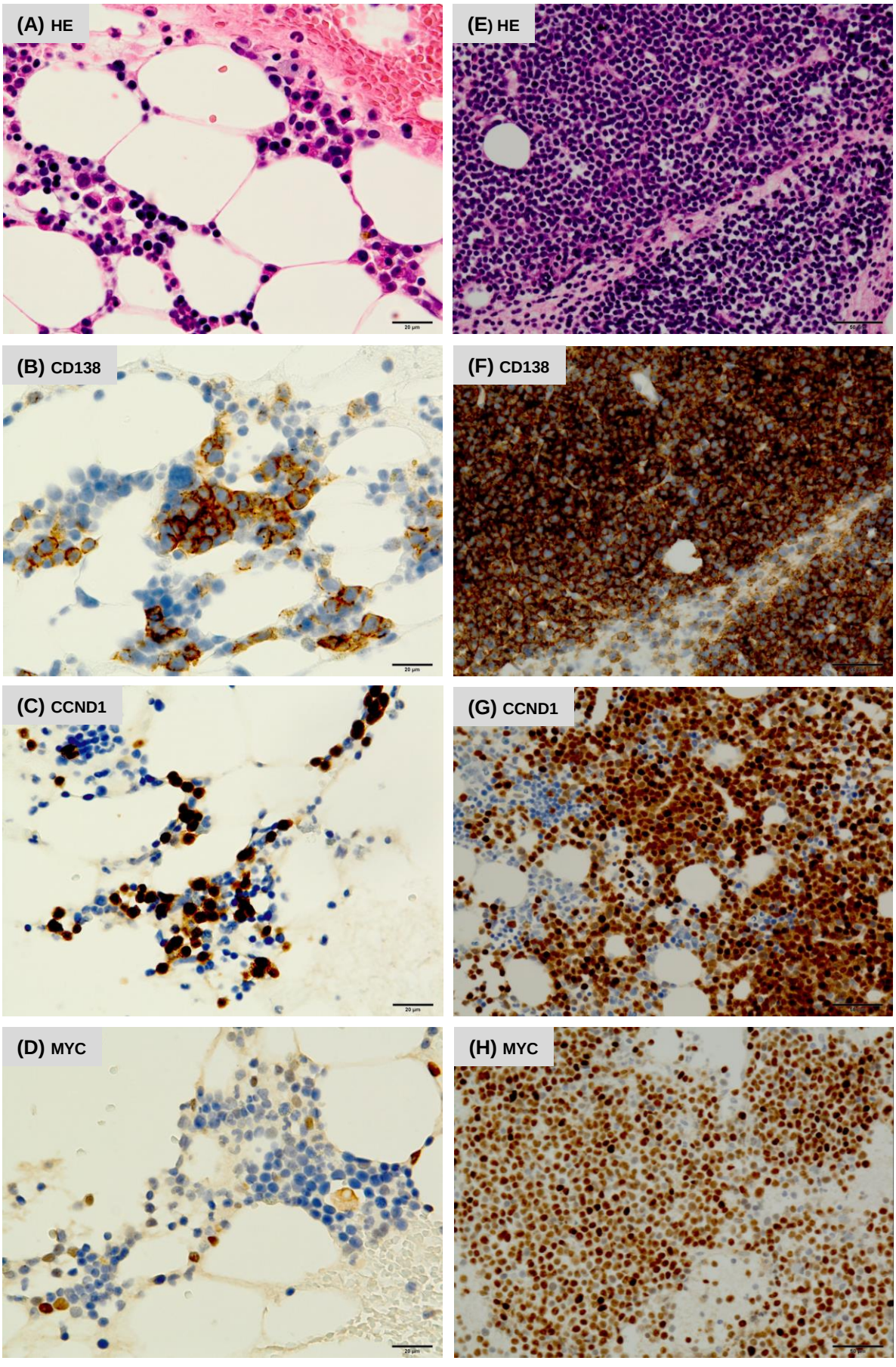
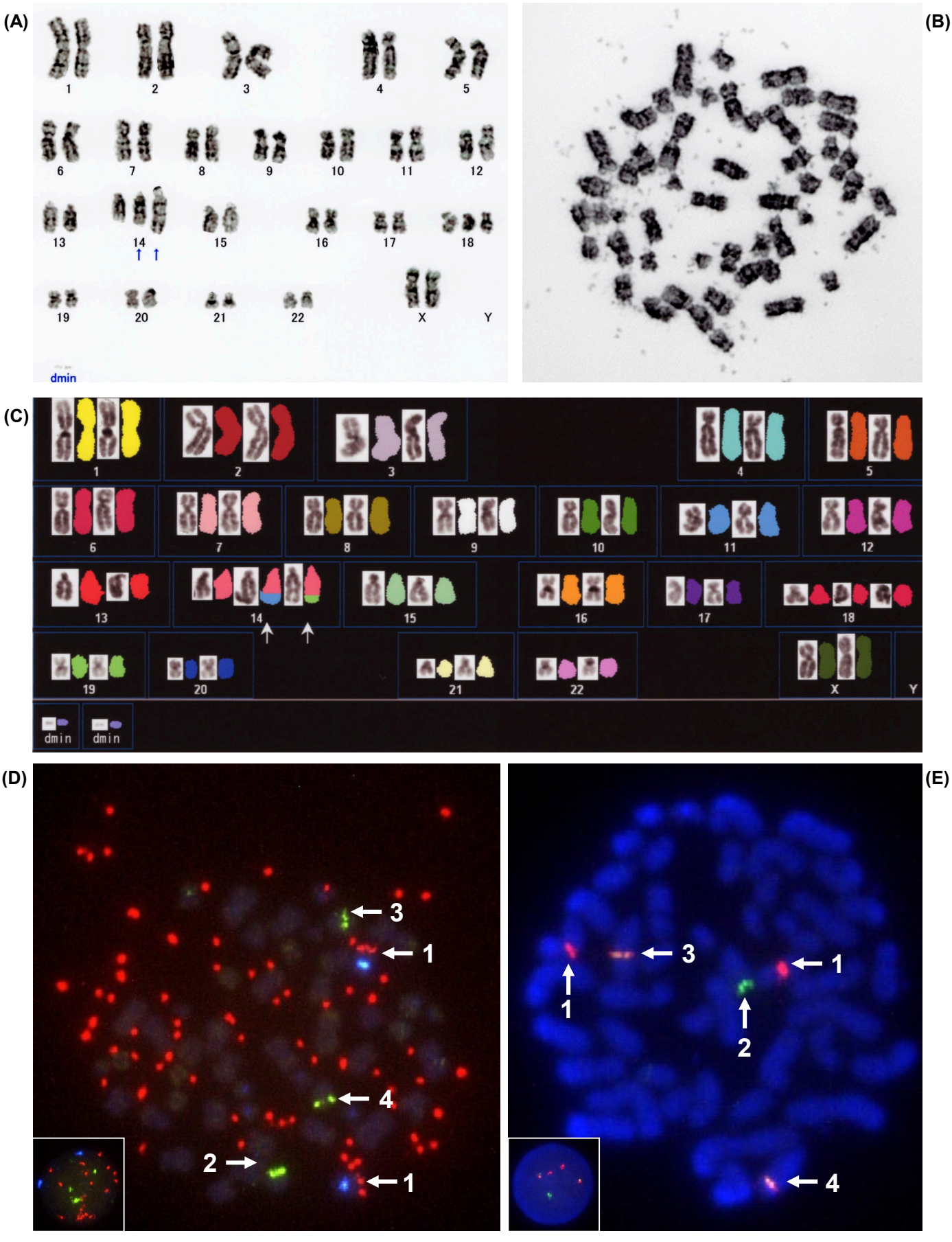


Fig. 3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