Amplification of the MYC gene in immunoglobulin light chain (AL) amyloidosis

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INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is characterized by tissue deposits of light chain protein as amyloid fibrils, leading to the dysfunction of vital organs.¹,² The disease is underlain by plasma cell neoplasms such as monoclonal gammopathy of undetermined significance, multiple myeloma (MM), or Waldenström macroglobulinemia.¹ In general, cytogenetic analysis of plasma cell neoplasms is challenging due to the low proliferative properties of neoplastic cells and metaphase spreads if obtained are often inadequate for karyotyping due to poor morphology.³ In MM, fluorescence in situ hybridization (FISH), in which fluorochrome-labeled probes designed to detect chromosomal translocations, deletions, or hyperdiploidy are applied to interphase nuclei instead...
of metaphases, has been substituted for conventional G-banding analysis. Currently, the FISH test has become a standard in diagnostic evaluation to categorize a patient into prognostic subgroups.

Cytogenetic abnormalities in AL amyloidosis determined by either or both conventional G-banding and interphase FISH are similar to those found in MM; however, predominant chromosomal abnormalities in AL amyloidosis have been described. In this report, we describe a female patient who presented with cardiac amyloidosis. Interphase FISH and G-banding applied to the bone marrow (BM) materials revealed the presence of the t(11;14)(q13;q32) translocation, which led to the generation of the CCND1-immunoglobulin heavy chain (IGH) fusion gene. We also found another recurrent abnormality involving chromosome 8 band q24 that contained a segment indicative of gene amplification.

CASE REPORT

Case Presentation

A woman in her sixties was admitted to the Department of Cardiology due to progressive dyspnea on exertion. Her chest X-ray demonstrated marked cardiomegaly, and atrial fibrillation and complete right bundle branch block were found on electrocardiography, in addition to thickening of the interventricular septum and ventricular wall associated with hypokinesis on echocardiography. Her hemoglobin level was 10.9 g/dL, white blood cell count was 5.73 \times 10^3/\mu \text{L}, and platelet count was 284 \times 10^3/\mu \text{L}. Her total serum protein level was 6.3 g/dL, albumin was 3.7 g/dL, globulin was 2.6 g/dL, lactate dehydrogenase was 345 IU/L, alkaline phosphatase was 1,070 IU/L, creatinine was 1.4 mg/dL, uric acid was 10.0 mg/dL, calcium was 9.4 mg/dL, and C-reactive protein was 0.06 mg/dL. The value of brain natriuretic peptide (BNP) was 1,604.2 pg/mL (reference, <18.4 pg/mL) and that of cardiac troponin T (cTnT) was 0.438 ng/mL (reference, <0.1 ng/mL). Endomyocardial biopsy of the posterior wall of the left ventricle revealed disruption of the muscle fibers by eosinophilic deposits that were positive on Congo red staining (Figure 1, top). After the resolution of hemorrhagic cardiac tamponade that developed after the biopsy procedure, the patient was referred to the Department of Hematology for hematological evaluation.

Serum protein electrophoresis demonstrated the hypogammaglobulinemia pattern with 9.2% \gamma globulin. The level of IgG was 648 mg/dL, IgA was 22 mg/dL, and IgM was 11 mg/dL. Her urine contained 20 mg/dL of protein. Although no M-protein was found in the serum by immunofixation, Bence-Jones protein (BJP)-\kappa was detected in the urine; the urine protein to creatinine (Cr) ratio that estimated the daily amount of urinary BJP excreted was 0.3 g/gCr. The serum free light chain (FLC-)\kappa level was 776.0 mg/L (reference range, 3.3 to 19.4 mg/L), FLC-\lambda was 9.9 mg/L (5.7 to 26.3 mg/L), and the \kappa/\lambda ratio was 78.38 (0.26 to 1.65). The \beta2-microglobulin level was 5.06 \mu g/mL. No hepatosplenomegaly or osteolytic lesions were found by imaging studies.

Examination of the BM aspirate smears revealed 25.6% plasma cells exhibiting small mature plasma cell morphology. Occasional cells had two or more nuclei (Figure 2, top). The cells were CD19\(^{-}\), CD20\(^{-}\), CD28\(^{-}\), CD38\(^{+}\), CD45RA\(^{-}\), CD56\(^{+}\), and CD138\(^{+}\), and had cytoplasmic immunoglobulin \kappa light chain restriction but lacked heavy chain expression by flow cytometry (Figure 3). BM biopsy specimens showed hypocellularity but confirmed spotted aggregates of CD138\(^{+}\) plasma cells with \kappa light chain restriction by immunohistochemistry (Figure 2, bottom). Skin biopsies from the abdomen revealed amyloid deposits in thickened walls of blood vessels (Figure 1, bottom).

Cytogenetic Evaluation

FISH of the BM smear slides with the CCND1/immunoglobulin heavy chain (IGH) dual-fusion translocation probe, composed of red-labeled CCND1 and green-labeled IGH probes, detected two red, one green, and one yellow (i.e. fusion) signal in 26.5% of the BM cell nuclei (Figure 4A), consistent with the
Figure 1. Microscopic examination of biopsies of myocardium (top) and skin (bottom). a, hematoxylin and eosin (H&E) staining; b, Elastica van Gieson staining; c, Congo red staining; d, H&E staining; e, Congo red staining; and f, Dylon staining. (Original magnification, ×20 objective lens).

Figure 2. Appearance of plasma cells in the BM (top) and histopathology of the BM biopsy (bottom). a, b, and c, Wright staining. The cells had basophilic cytoplasm and an eccentric nucleus with mildly condensed chromatin, and many of the cells had intracytoplasmic vacuoles. The perinuclear halo was inconspicuous. A multinucleated cell is shown in c. (Original magnification, ×100 objective lens). d, H&E staining (×10); e, anti-CD138 immunostaining (×20); and f, anti-immunoglobulin κ light chain (×20). The BM showed hypercellularity but contained spotted aggregates of CD138⁺ plasma cells with κ light chain restriction. No amyloid deposits were found in the BM.
Figure 3. Flow cytometry (FCM) of BM aspirates. The cells were first fixed and permeabilized by DAKO’s IntraStain kit and then subjected to multicolor FCM using antibodies against the indicated antigens. ‘c’ denotes cytoplasmic. In the top and middle, CD38⁺ and CD138⁺ plasma cells are colored in blue. The cells were negative for heavy chain expression (bottom).

percentage of plasma cells in the BM. Accordingly, G-banding karyotyping identified metaphase cells that had der(14)t(11;14)(q13;q32), whereas the reciprocal der(11)t(11;14)(q13;q32) was missing and instead had a normal chromosome 11 pair (Figure 4B). Additional FISH detected del(13)(q14) and del(17)(p13) in 28.4% and 26.8% of the nuclei, respectively.

G-banding revealed another recurrent abnormality involving chromosome 8 at band q24 that contained a homogeneous staining region (hsr) [der(8) (pter→q24:hsr::?)], in addition to the der(14)t(11;14)(q13;q32) (Figure 5). We then hybridized the metaphase spreads with the MYC dual-color probe, consisting of red-labeled 5′ MYC and green-labeled 3′ MYC probes. As a result, the normal chromosome 8 homologue was labeled with one yellow signal at 8q24, indicative of single copy of germline MYC, whereas multiple yellow signals were localized on the hsr of der(8), indicative of amplification of the MYC locus (Figure 5). We next counted MYC signals in the interphase nuclei of the chromosome preparation and found that the majority of the cells carried 2 or 3 copies of MYC, but only occasional cells had four or more MYC signals (Figure 6), suggesting that MYC amplification occurred in a fraction of neoplastic cells; the presence of metaphase cells with MYC amplification suggested that these cells were more proliferative in in vitro cell culture than those with 2 or 3 copies of MYC.

**Treatment Course**

The patient was diagnosed with AL amyloidosis with cardiac involvement in the setting of clonal proliferation.
Figure 4. FISH using the Vysis IGH/CCND1 XT DF FISH Probe (Abbott Laboratories, Abbott Park, IL, USA). (A) FISH of a BM smear slide. Pictures captured through TRITC (tetramethylrhodamine B isothiocyanate), FITC (fluorescein isothiocyanate), and DAPI/TRITC/FITC triple band-pass filters are aligned. Red, green, and yellow signals representing the CCND1, IGH, and CCND1-IGH fusion genes in a plasma cell nucleus are indicated by arrowheads of their respective colors. Three nuclei representing polymorphonuclear leukocytes carry two red and two green signals. (B) FISH of chromosome preparation. Pictures of TRITC, FITC, and triple band-pass filters and G-banding are aligned. Two copies of chromosome 11 labeled by a red signal, chromosome 14 by a green signal, and der(14)t(11;14)(q13;q32) by a yellow signal are indicated.

DISCUSSION

In general, plasmacytosis in BM in patients with AL amyloidosis is mild compared with that in patients with MM.\(^2,7\) In the present patient, however, as BM plasma cells were ≥10%, meeting the diagnostic criteria of MM,\(^12\) she could be considered to have AL amyloidosis with concomitant BJP-type MM.\(^13\) Nevertheless, as the patient first presented with cardiac symptoms, there were no myeloma-defining events at presentation, BM biopsy showed hypocellularity with spotted plasma cells aggregates, and the level of urinary excretion of BJP was low, the diagnosis of AL amyloidosis with cardiac involvement is preferable despite the unproportionally
**Figure 5.** Two metaphase spreads hybridized with the Vysis MYC Dual Color Break Apart Rearrangement Probe (Abbott Laboratories). The probe consists of red-labeled 5’ MYC and green-labeled 3’ MYC, and yellow (fusion) signal indicates unrearranged MYC. Pictures of TRTIC, FITC, and triple band-pass filters and G-banding are aligned. Chromosome 8 labeled by a yellow signal and der(8)(pter→q24::hsr::?) labeled by multiple yellow signals are indicated by thin and thick arrows, respectively. On the bottom, two nuclei labeled by either two or three fusion signals (arrowheads) are shown. Asterisks on the G-banding pictures indicate der(14) t(11;14)(q13;q32) chromosomes.

**Figure 6.** FISH of chromosome preparation showing the copy number of MYC in interphase nuclei and a metaphase spread. Occasional nuclei carrying 4 or more signals are indicated by arrows.
high plasma cell counts in the BM differential. Indeed, in a Mayo clinic series of AL amyloidosis, the percentage of BM plasma cells in the “AL amyloidosis without MM” group ranged from 2 to 26%, and the disease was defined as all forms of systemic amyloidosis originating from monoclonal light chains, regardless of the nature of the underlying plasma cell disorder.

Although no single cytogenetic abnormality that is diagnostic of AL amyloidosis has been recognized, when a FISH panel for the characterization of MM is applied to patients with AL amyloidosis, the t(11;14)(p13;q32)/CCND1-IGH translocation is the most common abnormality and is found in nearly half of these patients. Other common abnormalities include del(13)(q14) and the gain of 1q21, whereas del(17)(p13), indicative of the inactivation of TP53, is uncommon. On the other hand, amplification of the MYC gene has been described in relapsed/refractory MM patients and MM cell lines, suggesting that MYC amplification is a secondary genetic abnormality, and is related to disease progression and drug resistance. To the best of our knowledge, this is the first report of MYC amplification resulting from the generation of hsr in an AL amyloidosis patient carrying t(11;14)(q13;q32)/CCND1-IGH.

MYC is a potent proto-oncogene located on chromosome 8 at band q24 and the gene product functions as a transcription factor, whose activation leads to increased DNA replication and protein synthesis. As MYC amplification theoretically leads to increased levels of the MYC protein product, this genetic abnormality is likely involved in increased cell proliferation, thereby functioning in tumorigenesis. However, in this case, the cells with MYC amplification were found on initial presentation and accounted for a fraction of neoplastic cells that were clonally marked with t(11;14)(q13;q32)/CCND1-IGH. Thus, the presence of MYC amplification is unlikely to indicate disease progression or drug resistance, but instead represents intraclonal genetic heterogeneity in neoplastic plasma cells, consistent with large-scale sequencing analyses, and diverse subclones in terms of the copy number of MYC being present at presentation. Whether the cells with MYC amplification acquire a growth advantage to become predominant clones or whether they are eradicated by treatment to be replaced by more resistant clones remains to be elucidated.

REFERENCES
1. Rajikumar SV, Dispenzieri A. Clinical presentation, laboratory manifestations, and diagnosis of immunoglobulin light chain (AL) amyloidosis. In: Glassock RJ, Kyle RA, Connor RF, eds. UpToDate; 2019.
2. McKenna RW, Kyle RA, Kuehl WM, et al. Plasma cell neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). Lyon: IARC; 2017:241-258.
3. Nilsson T, Lenhoff S, Rylander L, et al. High frequencies of chromosomal aberrations in multiple myeloma and monoclonal gammopathy of undetermined significance in direct chromosome preparation. Br J Haematol 2004;126:487-494.
4. Boyd KD, Ross FM, Chiecchio L, et al. A novel prognostic model in myeloma based on co-segregating adverse FISH lesions and the ISS: Analysis of patients treated in the MRC Myeloma IX trial. Leukemia 2012;26:349-355.
5. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: Spotlight review. Leukemia 2009;23:2210-2221.
6. Chng WJ, Dispenzieri A, Chim CS, et al. IMWG consensus on risk stratification in multiple myeloma. Leukemia 2014;28:269-277.
7. Harrison CJ, Mazzullo H, Ross FM, et al. Translocations of 14q32 and deletions of 13q14 are common chromosomal abnormalities in systemic amyloidosis. Br J Haematol 2002;117:427-435.
8. Hayman SR, Bailey RJ, Jalal SM, et al. Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. Blood 2001;98:2266-2268.
9. Bochtler T, Merz M, Hielscher T, et al. Cytogenetic intraclonal heterogeneity of plasma cell dyscrasias in AL
amyloidosis as compared with multiple myeloma. Blood Adv 2018;2:2607-2618.
10. Bochtler T, Hegenbart U, Cremer FW, et al. Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared with monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability. Blood 2008;111:4700-4705.
11. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. J Clin Oncol 2012;30:989-995.
12. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538-548.
13. Kourelis TV, Kumar SK, Gertz MA, et al. Coexistent multiple myeloma or increased bone marrow plasma cells define equally high-risk populations in patients with immunoglobulin light chain amyloidosis. J Clin Oncol 2013;31:4319-4324.
14. Dib A, Gabrea A, Glebov OK, et al. Characterization of MYC translocations in multiple myeloma cell lines. J Natl Cancer Inst Monogr 2008:25-31.
15. Sekiguchi N, Ootsubo K, Wagatsuma M, et al. Impact of C-Myc gene-related aberrations in newly diagnosed myeloma with bortezomib/dexamethasone therapy. Int J Hematol 2014;99:288-295.
16. Dang CV. MYC on the path to cancer. Cell 2012;149:22-35.
17. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer 2012;12:335-348.
18. Walker BA, Leone PE, Chiecchio L, et al. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. Blood 2010;116:e56-65.
MYC遺伝子増幅を認めた免疫グロブリン軽鎖（AL）アミロイドーシスの一例

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症例は69代女性. 心病変を主病変とする免疫グロブリン軽鎖（AL）アミロイドーシスを発症した. ALアミロイドーシスは Mayoクリニック改訂リスク分類のⅣ期に該当した. 骨髄腫診断事象は認めなかった. 血清中には M 蛋白を認めなかっだが, 尿中に κ型ベンソジョーンズ蛋白を認めた. 骨髄は低形成であったが, スメア標本の細胞分類で形質細胞を25.6% 認め、免疫染色とフローサイトメトリーで κ 鎖軽鎖制限を認めた. FISH と G バンディングで t(11;14)(q13;q32) 転座と CCND1-IGH 融合遺伝子を認めた.一方, 8 番染色体 q24 バンドに homogeneously staining region を含む構造異常を認め, この領域には多数の MYC 遺伝子シグナルがクラスターしていた.しかし, 大半の間期核では, MYC のシグナルは 2 または 3 個で, 4 個以上のシグナルが認められたのは一部の核に限られていたことから, MYC 遺伝子増幅は腫瘍細胞の一部にみられたと考えられた. 従って, MYC 遺伝子増幅はクローン内多様性を示し, 腫瘍クローンが MYC 遺伝子のコピー数の異なる多様なサブクローンから構成されていることが示唆された.

キーワード： 免疫グロブリン軽鎖 (AL) アミロイドーシス, FISH, t(11;14)(q13;q32)/CCND1-IGH 転座, MYC 遺伝子増幅, クローン内多様性