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LETTER TO THE EDITOR

A myeloma cell line established from a patient refractory to thalidomide therapy revealed high-risk cytogenetic abnormalities and produced vascular endothelial growth factor

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Recent advances in the treatment of multiple myeloma (MM) using newly developed drugs, such as thalidomide, lenalidomide and bortezomib, have improved the survival of the patients with refractory disease. However, it remains difficult to treat certain populations of MM patients; these patients are considered high-risk cases. Tumor cells obtained from these patients conferred characteristic chromosomal abnormalities, including deletion of chromosome 13 (del 13), t(4;14) translocation and deletion of chromosome 17, on which tumor suppressor gene TP53 is localized.[1–4] Here we report the establishment of a novel myeloma cell line, MUM24, which was obtained from the bone marrow of a 64-year-old female patient with immunoglobulin G (IgG) (k)-type MM. She was refractory to melphalan plus prednisolone, vincristine plus doxorubicin plus dexamethasone and high-dose dexamethasone as well as thalidomide. A bone marrow sample was obtained in September 2002, and was used for establishing the MUM24 cell line. The patient passed away in December 2002 due to systemic progression of the MM, including intracranial invasion.

Microscopic pictures of MUM24 cells are provided in Figure 1a. Most of the cells were mononuclear but occasionally bi- or multinuclear cells were observed. The cells had irregularly shaped cytoplasm and vacuoles were observed in some cells. The karyotype analysis of MUM24 cells by Giemsa staining revealed complex chromosomal changes together with del 13 and other abnormalities (Figure 1b). The representative abnormalities were 45, X, add(X)(p22), add(1)(p11), del(1)(q32q42), add(2)(q11), del(2)(q31q33), add(4)(q21), i(5)(p10), der(6;11)(p10;p10), +8, −13, −14, add(16)(q22), add(17)(q25), del(17)(p11), der(21)t(1;21)(q11;p12), add(22)(q12), +mar. The fluorescence *in situ* hybridization (FISH) analysis showed that the p53 gene is deleted in the MUM24 cell line (Figure 1c). The t(4;14) fusion signals were also detected using fibroblast growth factor receptor 3 (FGFR3) and immunoglobulin heavy chain probes.

We also examined the genomic DNA sequences of residual TP53 gene of the MUM24 cells and found a nonsense mutation from 'tgg' to 'tga' at codon 273 in exon 4 (Figure 1d). Thus, no functional TP53 gene exists in MUM24 cells. The cell surface antigen characteristics for myeloma cells, such as CD38, CD56, Ia (DR) and CD138 were all positive in nearly 100% of the MUM24 cells. B-cell antigens, CD19, CD20 and cell-surface IgG were negative. Cereblon (CRBN), a component of the E3 ubiquitin ligase complex, was identified as a thalidomide-binding protein, which is considered to be a responsible molecule for thalidomide-induced teratogenicity.[5] CRBN is also known as a critical target for the antimyeloma effect of lenalidomide and pomalidomide. In MUM24 cells, CRBN transcripts were detected (data not shown). The involvement of CRBN expression in thalidomide resistance is still unclear.

It was reported that bone marrow angiogenesis have a role in the progression of myeloma cells.[6] We previously demonstrated that MM cells produce and secrete angiogenic growth factors such as fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF).[7] The plasma concentrations of these growth factors in MM patients are associated with disease activity and prognosis.[7] Thus, we examined the concentrations of these growth factors in the culture medium of MUM24 cells. An enzyme-linked immunosorbent assay showed that MUM24 cells (5×10^5/ml) secreted large amounts of VEGF (1.82 μg/ml). FGF-2 (8.21 ng/ml) and HGF (2.67 μg/ml) were also detected in the culture medium. We also conducted reverse transcription-PCR to detect the expression of FGFs and their receptor family. The MUM24 cells expressed FGF-1, -2 and -4 as well as FGFR1, 2, 3 and 4 (data not shown). In addition, as reflected by the FISH analysis results, t(4;14) translocation resulted in the expression of FGFR3. We therefore speculate that angiogenic growth factors also mediate the interaction of MM cells with bone marrow interstitial cells. The microenvironment in the bone marrow niche may protect MM cells from exogenous noxious stimuli such as antimyeloma drugs including thalidomide.

In order to examine the response of MUM24 cells to the newly developed drugs, we incubated MUM24 cells with various concentrations of thalidomide, lenalidomide or bortezomib, and then evaluated the growth suppression (Figure 1e). As a reference, KMS-21 cells (which do not have high-risk chromosomal changes such as t(4;14) or del 13 and 17) were also used. MUM24 cells did not respond to thalidomide even at concentrations that were much higher than therapeutic plasma concentrations *in vitro* (1–10 μM)(data not shown). Lenalidomide and bortezomib inhibited the growth of MUM24 cells in slightly higher concentrations compared with KMS-21 cells. Namely, the IC_{50} values of lenalidomide and bortezomib for the MUM24 cells were over 25μm and 3.5 nM, whereas those for the KMS-21 cells were ~3 μM and 2.4 nM, respectively.

To examine tumorigenicity, 1×10^7 MUM24 cells were subcutaneously injected in 5-week-old male NOD/Shi-SCID, IL-2Rg KO Jic (NOG) mice, and skin plasmacytomas were established in 2 weeks. Macroscopic and microscopic pictures of a typical skin plasmacytoma are shown in Figure 2a and b. Plasma cells with eccentric oval nuclei with prominent nucleoli were significantly proliferated. Immunohistochemical staining showed that the tumors were positive for CD138 staining (Figure 2c). To evaluate tumor angiogenesis, we also stained endothelial cells, and we found that CD34-positive endothelial cells proliferated in the tumors (Figure 2d).

Taken together, the results of the present study demonstrate that the MUM24 cell line, established from a thalidomide-resistant MM patient, harbored high-risk cytogenetic changes and produced VEGF. Further studies using MUM24 and other MM cells on the expression of multiple myeloma SET and CRBN proteins, intracellular signaling via angiogenic growth factors and antitumor activities of immunomodulatory drugs *in vivo* are

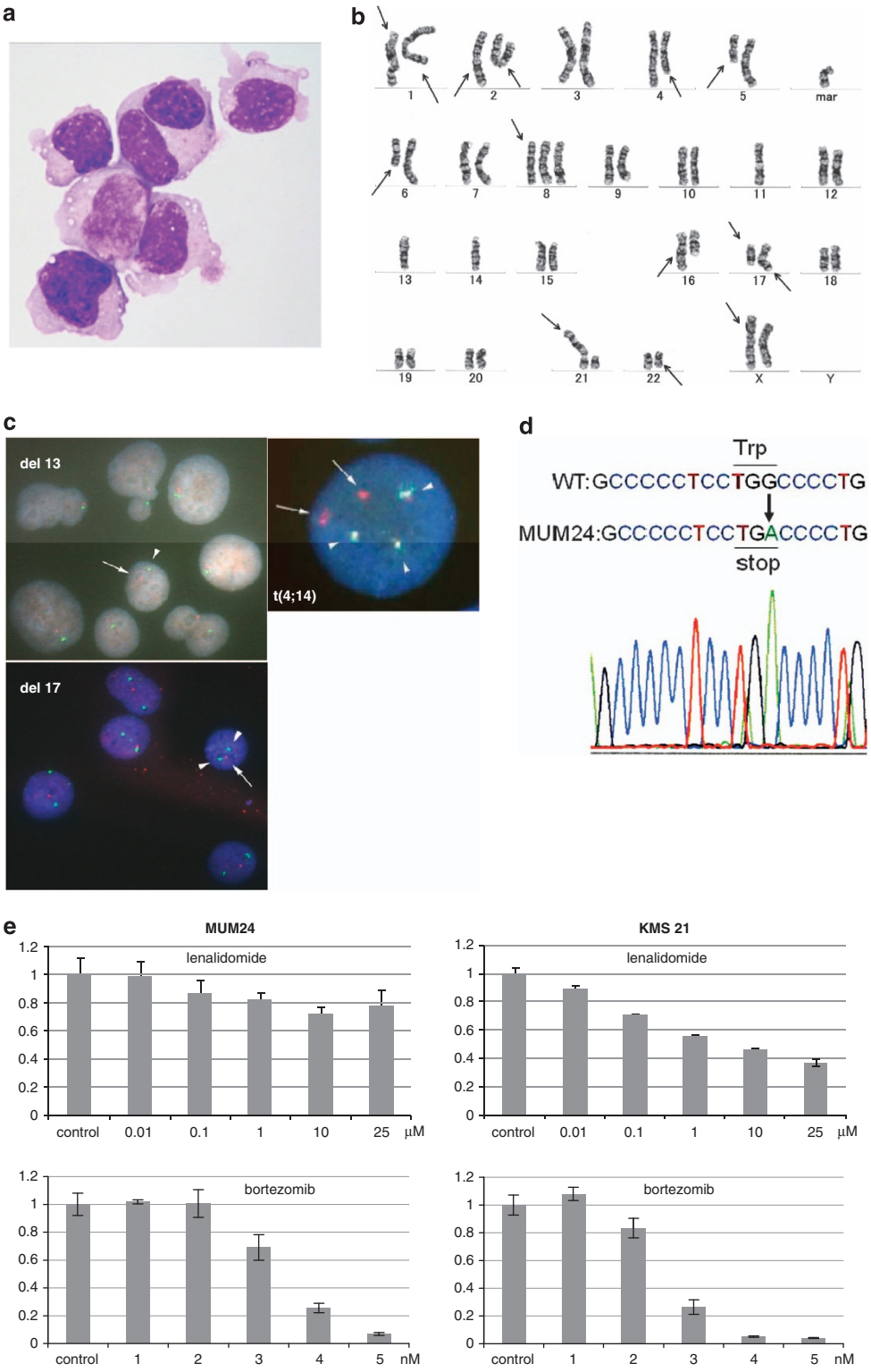

Figure 1. Morphological and biological characterization of MUM24 cells. May-Gruenwald-Giemsa-stained cytospin specimens are shown (**a**). Typical chromosomal aberration (**b**). Multicolor FISH analyses were performed using RB1 (13q14) red, LEU (13q14) red and PIXB (13q34) green probes for the detection of deletion of chromosome 13; FGFR3 (4p16.3) red and IGH (14q32) green probes for t(4;14) translocation; and p53 (17p13.1) red and CEP 17 (Cen) green probes for the deletion of chromosome 17 (**c**). Genomic DNA sequence of TP53 gene of MUM24 cells. 'TGG' was mutated to 'TGA' nonsense mutation at the codon 273 in exon 4 (**d**). Growth inhibition of MUM24 cells by newly developed drugs. MUM24 and KMS-21 cells were incubated with various concentrations of lenalidomide or bortezomib for 48 h (**e**).

necessary to elucidate molecular mechanisms for thalidomide resistance. As mentioned earlier, overcoming high-risk myeloma is a present unmet clinical need. MUM24 cells are expected to be useful as a tool for studying the mechanisms of thalidomide resistance, as well as for the screening of novel drugs to overcome high-risk MM.

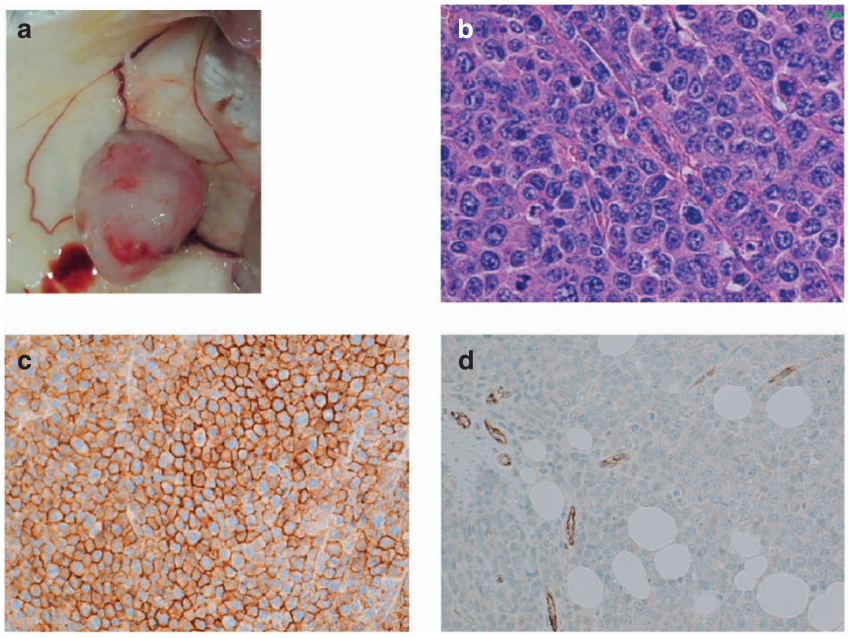

Figure 2. Xenograft model in mice: 1×10^7 MUM24 cells were inoculated subcutaneously in NOD/Shi-scid, IL-2R SCID (NOG) mice. Macroscopic (**a**) and microscopic (**b**) pictures of subcutaneous plasmacytomas. Immunohistological staining of CD138 antigen (**c**). Microvessels using antibody against mouse anti-von Willebrand factor (**d**).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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