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ORIGINAL ARTICLE

Double minute chromosomes in acute myeloid leukemia, myelodysplastic syndromes, and chronic myelomonocytic leukemia are associated with micronuclei, *MYC* or *MLL* amplification, and complex karyotype

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Double minute chromosomes (dmin) are small, paired chromatin bodies that lack a centromere and represent a form of extrachromosomal gene amplification. Dmin are rare in myeloid neoplasms and are generally associated with a poor prognosis. Most studies of dmin in myeloid neoplasms are case reports or small series. In the current study, we present the clinicopathologic and cytogenetic features of 22 patients with myeloid neoplasms harboring dmin. These neoplasms included acute myeloid leukemia (AML) (n = 18), myelodysplastic syndrome (MDS) (n = 3), and chronic myelomonocytic leukemia (CMML) (n = 1). The AML cases consisted of AML with myelodysplasia-related changes (n = 13) and therapy-related AML (n = 5). Dmin were detected in initial pre-therapy samples in 14 patients with AML or CMML; they were acquired during the disease course in 8 patients who had AML or MDS. The presence of dmin was associated with micronuclei (18/18; 100%), complex karyotype (17/22; 77.3%), and amplification of *MYC* (12/16; 75%) or *MLL* (4/16; 25%). Immunohistochemical staining for MYC performed on bone marrow core biopsy or clot sections revealed increased MYC protein in all 19 cases tested. Except for one patient, most patients failed to respond to risk-adapted chemotherapies. At last follow up, all patients had died of disease after a median of 5 months following dmin detection. In conclusion, dmin in myeloid neoplasms commonly harbor *MYC* or *MLL* gene amplification and manifest as micronuclei within leukemic blasts. Dmin are often associated with myelodysplasia or therapy-related disease, and complex karyotypes.

Keywords Double minute chromosomes, acute myeloid leukemia, myelodysplastic syndrome, micronuclei, *MYC*, *MLL*

Introduction

Double minute chromosomes, or double minutes (dmin), are small, paired, usually spherical chromatin particles that represent a form of extrachromosomal gene amplification. Although dmin are found in a variety of human cancers (1,2) their presence in hematologic malignancies is unusual, with an overall frequency of ~1% (3–7). Dmin have been reported to be associated with rapid disease progression and short survival in

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acute myeloid leukemia (AML) (8,9). They are more common in primary (*de novo*) AML than in secondary AML, often associated with a complex karyotype (9). The most commonly amplified gene in dmin is *MYC*. Other less commonly amplified genes include *MLL*, *REL*, *C-ETS 1*, and *Htrx-1* (9–14). A subset of hematologic malignancies with *MYC* amplification also have deletion of all or part of the long arm of chromosome 8, which may contribute to extra-chromosomal *MYC* amplification (15). Another feature of AML with dmin and *MYC* amplification is their budding or expulsion from the nuclei of leukemic blasts to form intracytoplasmic micronuclei that are visible by light microscopy (16,17).

Currently the literature on dmin in myeloid malignancies is limited to case reports or small case series, some with literature review (9,18,19). In this study, we present the clinical, pathological, and cytogenetic features of the largest cohort of patients with myeloid neoplasms harboring dmin from a single institution.

Materials and methods

Case selection

We searched the database of the Clinical Cytogenetics Laboratory in the Department of Hematopathology at The University of Texas MD Anderson Cancer Center for cases of myeloid neoplasms with dmin in at least two metaphases over a 16-year period (January 1, 1998, to July 30, 2014). The diagnoses were based on the criteria of the French–American–British (FAB) (20) and the World Health Organization (WHO) classification systems (21). The clinical data, including treatment, response, and outcome, were obtained from the medical records.

Morphologic, cytochemical, and immunohistochemical examinations

For all cases, we reviewed formalin-fixed, paraffin-embedded decalcified bone marrow (BM) core biopsy and clot sections stained with hematoxylin and eosin. We performed 500-cell manual differential counts on Wright–Giemsa-stained aspirate smears and/or touch imprints in all cases. For 18 cases with material available for review (cases 1–3, 5–8, 10–14, 16, 18–22), blasts were examined for the presence of micronuclei. Cytochemical staining for myeloperoxidase was performed on BM aspirate smears in cases with more than 20% blasts using a standard method.

Immunohistochemical staining (IHC) for MYC was performed using a pre-diluted rabbit monoclonal antibody specific for MYC (clone Y69, Ventana Medical Systems, Tucson, Arizona) according to the manufacturer's recommendations. To determine the baseline level of MYC expression in the bone marrow, we also evaluated 11 negative staging BM samples from patients with diagnosis with lymphoma elsewhere without bone marrow involvement; all had less than 5% MYC-positive cells (data not shown). MYC expression was assessed semi-quantitatively by counting at least 1000 cells and determining the percent of MYC-positive cells in increments of five percentage points. Any case with 5% or more MYC-positive cells was considered positive.

Conventional cytogenetic and fluorescence in situ hybridization analyses

Conventional cytogenetic analysis was performed on G-banded metaphases from BM aspirates cultured for 24 and 48 hours without mitogen stimulation using standard techniques. Twenty metaphases were analyzed, and the results were reported using the International System for Human Cytogenetic Nomenclature. By definition, a complex karyotype has 3 or more karyotypic abnormalities. Fluorescence in situ hybridization (FISH) analysis was performed in all cases on BM cultures (2 cases), BM aspirate smears (7 cases), or FFPE clot sections (13 cases) using dual-color break-apart probes for *MYC* and *MLL*, according to the manufacturer's instructions (Vysis/Abbott Laboratories, Des Plaines, IL).

Results

Clinical findings

The clinicopathologic characteristics of the 22 patients are summarized in Table 1. We identified 22 cases of MDS, CMML, or AML with dmin over a period of 16 years. There were 12 men and 10 women, with a median age of 66 years (range, 24–84 years). Six patients (cases 14–18, 22) had a prior history of cytotoxic therapy for a non-myeloid malignant neoplasm. Prior malignancies included breast cancer (cases 14, 16), plasma cell myeloma (case 15), testicular cancer (case 17), prostate cancer and chronic lymphocytic leukemia (case 18), and urothelial cancer (case 22). Cytotoxic therapies included radiation therapy (cases 14, 16–18) and chemotherapy (cases 15 and 22). The interval from the initiation of cytotoxic therapy to the diagnosis of myeloid malignancy ranged from 2 to 38 years (median, 6 years).

Morphologic features and immunohistochemical findings for MYC

Notably, all cases showed myelodysplastic changes, and micronuclei were seen in blasts in all cases examined (Figure 1A–C). According to WHO criteria, 18 patients (cases 1–18) were classified as AML, 13 as AML with myelodysplasia-related changes (AML-MRC) and five as therapy-related AML (t-AML). Three patients (cases 19–21) were classified as MDS (refractory anemia with excess blasts, RAEB-1) and one (case 22) as therapy-related chronic myelomonocytic leukemia (t-CMML). Of the 13 patients with AML-MRC, the diagnosis in five was based on the presence of a complex karyotype (and dysplasia); the diagnosis in eight was based on the presence of multi-lineage dysplasia. Based on FAB criteria, 13 cases were classified as AML (cases 1–8, 11, 14–17), eight as MDS (RAEB or RAEB-T), and one as CMML. The subtypes of AML were M2 (n = 5), M1 (n = 4), M4 (n = 3), and M6 (n = 1) (Table 1).

Immunohistochemical staining for MYC was performed on 19 cases with available material available. All cases were positive for MYC expression (Figure 1D–F). MYC-positive cells ranged from 15% to 90% (median, 60%) of total marrow cellularity (Table 1).