A case of acute myeloid leukemia with gain of two copies of neocentromeric chromosome 11

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Background

Neocentromeres are ectopic centromeres that arise on a chromosome at a location that is normally not centromeric [1]. Since the discovery of the first human neocentromere in 1993, more than 90 cases of constitutional supernumerary marker chromosomes containing neocentromeres have been documented. However, neocentromere formation has only been observed in three tumor categories including lipomatous tumors, lung carcinoma and acute myeloid leukemia (AML) [2]. In AML, neocentromeric chromosomes have been described in three cases: a derivative r(8) [3], inv dup(10q) [4] and neo(1)[5].

Clinical Presentation

Patient was an otherwise healthy 76-year-old woman with a relatively unremarkable past medical history characterized by essential thrombocythemia diagnosed 17 years ago. Patient presented to clinic due to intermittent sweats and weight loss with a white cell count of 10.4, a hemoglobin of 12 g/dL, and a platelet count of 189,000 with an estimated 28% circulating blasts. The morphologic evaluation showed numerous circulating blasts with a background of mature segmented neutrophils. Flow cytometry revealed a distinct population of CD-34-positive myeloeblasts with aberrant CD7 and dim CD4 expression. The findings were compatible with a diagnosis of AML.

Method

A core biopsy was obtained for cytogenetics analysis. Cells were cultivated for 24 hours without mitogenic stimulation and then Colcemid was added to a final concentration of 0.05 µg/mL for 20 minutes. The cells were exposed to a hypotonic solution (0.075 mol/L KCl) for 20 minutes at 37 °C and then fixed three times in fresh Carnoy's fixative. Slides were prepared by use of a drying chamber. Preparations were banded with the GTG banding technique. Totally twenty cells were fully analyzed. Clonal karyotypic abnormalities were identified and described according to the 2016 International System of Human Cytogenetic Nomenclature.

Fluorescence in situ hybridization (FISH) was performed on previously analyzed banded metaphase nuclei using a cocktail of LSI *MLL* labeled Spectrum Orange and Spectrum Green colocalized at 11q23 (Abbott Molecular, Des Plaines, IL) and *CCP11* (D11Z1) labeled orange (CytoTest Inc., Rockville, MD) combined in IntelliFISH hybridization buffer (Abbott Molecular). Slides were destained using 3:1 methanol: glacial acetic acid and washed with 2xSSC, followed by sequential dehydrating ethanol series (70%, 85%, and 95%). Cells and probe were codenatured by heating at 80°C for 2 minutes using a ThermoBrite instrument (Abbott Molecular). Hybridization was performed overnight at 37°C. Finally, the slides were mounted with Vectashield containing DAPI (Vector Laboratories). Localization of the probes was confirmed on pooled cytogenetically normal blood controls.

Immuno-FISH on elongated chromosomes was performed to validate the putative structure of neocentromeres. Slides were processed for immunocytochemistry as previously described (6). Briefly, slides were rinsed three times for 3 min each in 1X TEEN buffer and then blocked with 0.1% Triton X and 0.1% BSA overnight at 4°C. Primary antibodies (CENP-C and ACA) were applied and incubated in a humidified chamber at RT x 1h, and then rinsed three times for 4 min each in KB buffer. After drying, Alexa Fluor secondary antibodies were applied and incubated in a humidified chamber at RT for 30 min. Slides were rinsed three times for 4 min each in KB buffer and then counterstained with DAPI.

Results

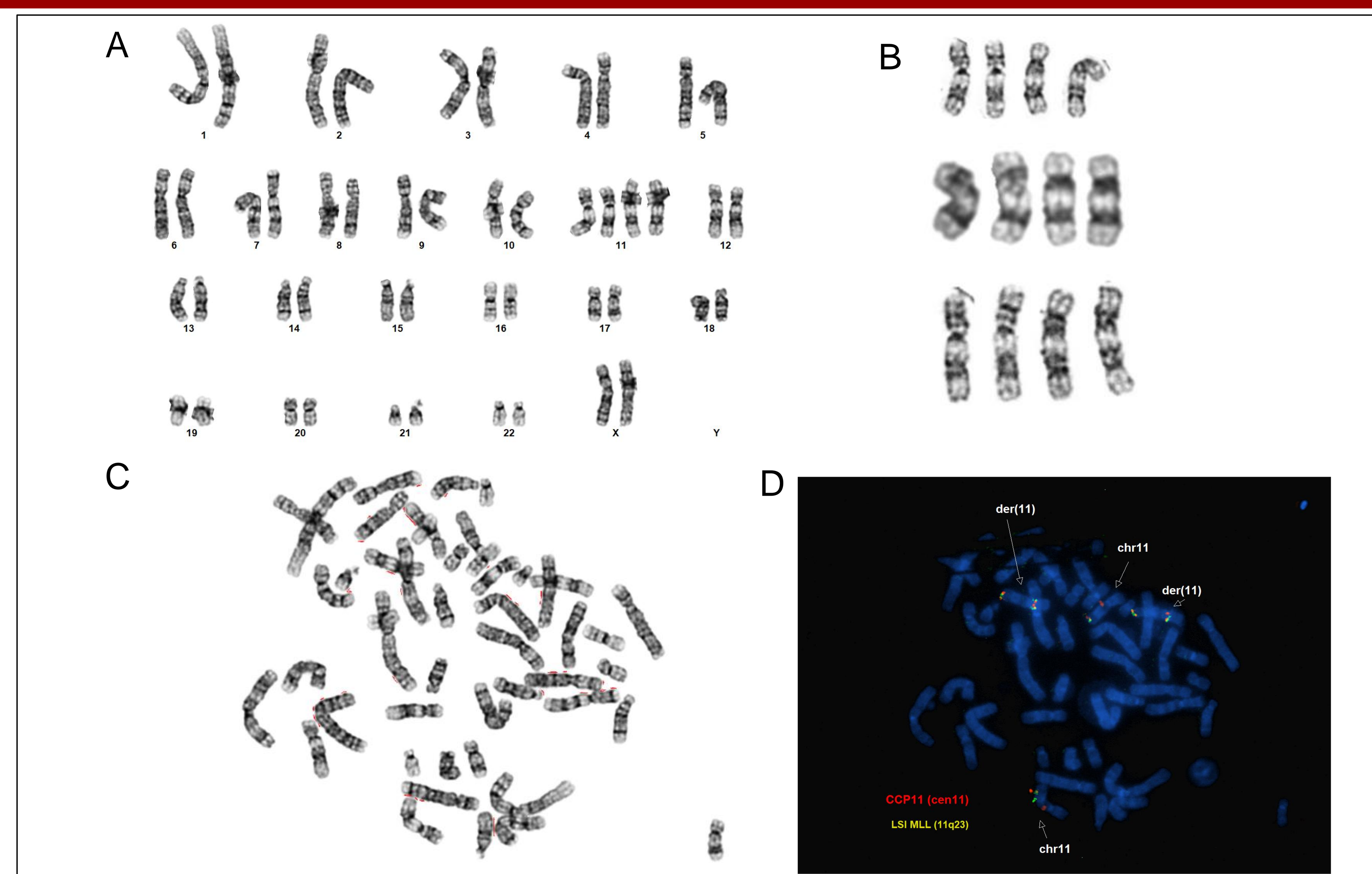

Figure 1 Cytogenetic analysis result. A) Representative karyotype of the abnormal clone with 48,XX,+11,+11,neo(11)(qter->q22->neo->q22->q13::q13->qter) B) Reflex image for neo(11); C) Corresponding metaphase; D) FISH result using a cocktail of LSI *MLL* labeled Spectrum Orange and Spectrum Green colocalized at 11q23 and *CCP11* (D11Z1) labeled orange

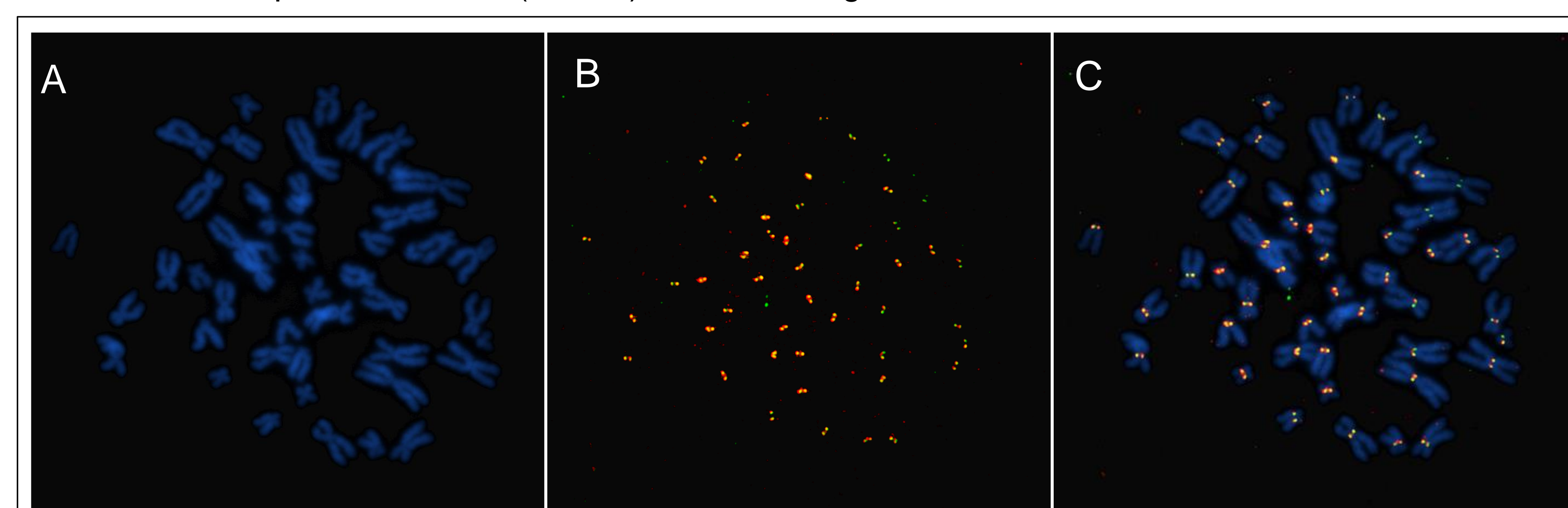

Figure 2 Immuno-FISH result to demonstrate all the chromosomes carry functional centromeres.A) DAPI staining; B) co-staining using anti-CENPC and anti-ACA antibody to localize the active centromere C)combined image

Case Summary

Here we report a neocentromeric chromosome 11 in a 76 year-old female with AML. Chromosome analysis revealed the karyotype 48,XX,+11,+11,neo(11)(qter->q22->neo->q22->q13::q13->qter)x2[19]/46,XX[1]. There are four copies of chromosome 11 in the abnormal clone, two structurally normal and two structurally abnormal (neo 11); and this leads to a total of six copes of 11q. FISH after G-banding confirmed the these two neocentromeres do not have the genomic sequence of centromere 11. Immuno-FISH on elongated chromosomes further validate the putative structure of neocentromeres.

Partial gain of chromosome 11q, containing the unrearranged mixed lineage leukaemia (MLL) gene is a rare but recurrent anomaly in myeloid malignancies. However, gain of 11q in the form of a neocentromeric chromosome 11 has not been described previously. Further study on the interaction between kinetochore protein and this active neocentremere will provide evidence about the mechanism of a functional neocentromere formation.

Reference

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