A practical quick staining method using hydrochloric acid-fast metachromatic dye for megakaryocytes

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Abstract—A specific stain using violet polymethine dye (VPM stain) for megakaryocytes was first developed by Kass (1995). We have modified this method for practical use in bone marrow specimens. The modified VPM stain labels megakaryocytes very well, while other marrow cells are poorly colorized. This staining procedure was more stable, and its color intensity was finer and clearer than the original. Using this stain, morphologic classification of megakaryocytes in bone marrow specimens from 11 normal and 8 myelodysplastic syndrome (MDS) patients was performed. Many megakaryocytes observed in MDS patients were juvenile compared with normal subjects according to their morphology. Blasts from acute megakaryoblastic leukemia (M7) and from a megakaryoblastic cell line (Mo7e) were also clearly stained with our method. This staining method is practical and very useful for rapid identification of megakaryocyte distribution and morphology.

Key words: Laboratory diagnostics; megakaryocytes; specific stain.

INTRODUCTION

In 1995, Kass [1] developed a megakaryocyte-specific stain using hypochlorite-fast metachromatic violet asymmetrical polymethine dye characterized chemically as 2-[4-(N-methyl-p-phenetidinyl)phenyl]-ethenyl-6-methoxycarbonyl-1,3,3-trimethyl-3H-indolium chloride (VPM chloride) (Fig. 1). This method stains megakaryocytes rose-pink with metachromasia, while other hematopoietic cells appear pale. Problems in the performance of this staining method prompted us to develop modifications of the original method. Our modified method was used to investigate marrow specimens obtained from patients with myelodysplastic syndrome (MDS), chronic myelocytic leukemia (CML), essential thrombocythemia (ET) and...
Figure 1. Structural formula of 2-[4-(N-methyl-p-phenetidinyl)-phenyl]-ethenyl-6-methoxycarbonyl-1,3,3-trimethyl-3H-indolium chloride.

polycythemia vera (PV), as well as cell lines (Mo7e) derived from acute megakaryoblastic leukemia and CD34\(^+\) stem cells committed toward megakaryocyte differentiation.

**MATERIALS AND METHODS**

*Specimens, patients and controls*

We examined 11 healthy volunteers and 58 patients with hematologic disorders (8 MDS, 1 CML, 2 ET, 2 PV, 1 Mo, 3 M1, 5 M2, 3 M3, 2 M5, 1 M6, 1 M7, 4 L1, 5 L2, 1 macroglobulinemia, 6 multiple myelomas, 7 non-Hodgkin’s lymphomas, 1 Hodgkin’s disease, 3 adult T-cell leukemias and 2 secondary thrombocytopenias due to hypersplenism). All were studied in the first bone marrow aspirate before treatment.

Bone marrow aspiration was performed at the posterior iliac crest. Smears were prepared in the normal manner and sent to the routine laboratory for diagnosis and to the experimental laboratory for our study. All testing was completed within 10 days after sample preparation.

*Cell line specimens*

(1) Mo7e, \((1 \times 10^4 \text{ cells/ml})\), was cultured in medium of RPMI 1640 (Gibco BRL, Grant Island, NY, USA) containing 10% fetal calf serum (FCS) and 5 ng/ml recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF) in a CO\(_2\) incubator (5% CO\(_2\)) with high humidity at 37\(^\circ\)C. Mo7e is a GM-CSF-dependent subline of Mo7. Mo7 is commonly used as a model for megakaryocytes as well as factor dependent cells [2–3].

(2) Preparation of hematopoietic progenitors with megakaryocytic differentiation. Peripheral blood stem cells were obtained from 4 patients with malignant lymphoma in complete remission after administration of chemotherapy drugs. Leukapheresis with the Fenwal CS-3000 Plus (Baxter, Deerfield, IL, USA), followed by Isolex (Baxter) treatment was used for purification of CD34\(^+\) cells [4, 5]. The isolated CD34\(^+\) cells were examined for their ability to differentiate into myeloid, erythroid, and megakaryocytic cells [6] as examined by Warren et al. [7]. The isolated CD34\(^+\) cells \((1 \times 10^4 \text{ cells/ml})\) were cultured in RPMI-1640 medium containing 10% FCS.