Laboratory techniques

Use of monoclonal anti-actin as a megakaryocyte marker in paraffin wax embedded bone marrow biopsy specimens

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SUMMARY Monoclonal anti-actin was used as a marker of megakaryocytes in Zenker's fixed, paraffin wax embedded bone marrow tissue, using an immunoperoxidase staining method. Twenty bone marrow samples were studied, including controls, and different myeloproliferative and myelodysplastic syndromes. The results were compared with those obtained using factor VIII related antigen (F VIII RAg) immunolabelling.

Anti-actin is as good a marker for megakaryocytes as anti-FVIIIIRAg and is potentially clinically useful when morphological identification is difficult, when bone marrow aspiration is unsuccessful, or when quantitative evaluation of tissue sections is required.

Well differentiated and mature megakaryocytes are easily detected in bone marrow biopsy specimens but the identification of more primitive megakaryocytes may be impossible by conventional methods. Immunohistochemical labelling of bone marrow biopsy specimens is of particular interest in such cases.

Most of the antibodies that can detect megakaryocytes are not suitable for routine bone marrow biopsy specimens,^{1,2} because they recognise antigens destroyed during fixation, decalcification, and paraffin wax embedding. Antibodies to factor VIII related antigen (FVIIIIRAg), however, have been used as markers of megakaryocytes in paraffin wax embedded bone marrow biopsy specimens and other tissue samples.^{3,4} In this paper we describe the use of an alternative monoclonal anti-actin with an immunoperoxidase method and compare the results between FVIIIIRAg and actin immunolabelling in normal bone marrows and in several haematological abnormalities which affect megakaryocytes.

Material and methods

Of the 20 patients studied, four had normal bone marrow biopsy specimens (taken for the staging of solid tumours) and 16 had different haematological

disorders affecting megakaryocytopoiesis: primary thrombocythemia (PT) n = 2; polycythemia rubra vera (PRV) n = 2; idiopathic myelofibrosis (MIF) n = 4; chronic granulocytic leukaemia (CGL) n = 4; and myelodysplastic syndrome (MDS) n = 4.

Bone marrow biopsy specimens were obtained by a conventional needle biopsy technique, immediately fixed in Zenker's solution, and embedded in paraffin wax after decalcification in acetic acid.

Immunoperoxidase staining using a polyclonal antibody to human FVIIIIRAg at a 1/800 dilution (Dakopatts, Denmark) and a monoclonal antibody to actin at a 1/2000 dilution (Amersham, UK) was done by the avidin-biotin method (Vectastain ABC Kit, Vector Laboratories, Burlingame, California, USA). Control samples were incubated with normal serum instead of primary antibody.

The results were evaluated by light microscopy, noting both the numbers of stained megakaryocytes and the intensity of their staining.

Results

Results of the immunoperoxidase staining for monoclonal actin and for polyclonal FVIIIIRAg are summarised in the table.

Selective staining of labelled megakaryocytes is shown beside the haematoxylin and eosin stain in the figure.

Figure Normal bone marrow. (a) Haematoxylin and eosin stain; (b) immunoperoxidase stain with monoclonal anti-actin; (c) immunoperoxidase stain with polyclonal anti-FVIIIIRAg.

Discussion

Actin is the principal protein of the platelet cytoskeleton and represents around 35% of the cell total protein content.⁵ It is synthesised by megakaryocytes and constitutes the contractile apparatus which regulates the shape changes occurring during activation of platelets.⁶

In our hands, actin proved a reliable marker for the megakaryocyte cell line in bone marrow samples, though this is, to the best of our knowledge, the first time the technique has been reported. Although it is known that many bone marrow cells contain actin⁷ only megakaryocytes are prominently stained by the method. Other structures that also stain for actin in bone marrow samples are muscle wall of the vessels, pericytes of the capillaries, and platelets.⁸

Megakaryocyte immunolabelling is clinically useful when difficulty arises in the morphological recognition of megakaryocytes because of immaturity and atypia, or when bone marrow aspiration is unsuccessful (dry tap), and when it is helpful to quantitate the megakaryocyte population accurately in tissue sections.

The intensity of the stain seems to depend on the degree of maturity and capacity of megakaryocytes for protein synthesis. Further studies should be done to evaluate the stage of megakaryocyte differentiation needed to stain with actin as has been previously done for FVIIIIRAg.^{9,10}

Actin is as good a marker for megakaryocytes as FVIIIIRAg, and it can be helpful when a study of megakaryocytes is needed on paraffin wax embedded bone marrow samples.

Table Results of immunoperoxidase staining for monoclonal anti-actin and for polyclonal anti-FVIIIIRAg antibodies

Diagnosis	No of cases	Actin	FVIIIIRAg
Control	4	++, +++, +++, ++++	++++, +++, +++, ++++
PT	2	++++, ++++	-, +
PRV	2	++++, -	++++, +
MF	4	+, -, +++, ++++	++++, -, +, ++++
CGL	4	+, +, +++, ++++	++++, +++, +++, ++
MDS	4	+, +++, +++, ++	+, +++, +++, +

++++ = strong or +++ = moderate positivity in more than 75% of megakaryocytes.
 ++ = strong or + moderate positivity in between 25% to 75% of megakaryocytes.
 - = less than 25% of megakaryocytes stained or a very weak positivity considered to be negative.

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