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 myelodysplastic and myeloproliferative disorders affecting megakaryocyte biology: primary thrombocytopenia (PT) n = 2; polycythaemia rubra vera (PRV) n = 2; idiopathic myelofibrosis (MF) 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 haematotoxylin and eosin stain in the figure.

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

Discussion

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

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 actin 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.

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 FVIIIRAg.

Actin is as good a marker for megakaryocytes as FVIIIRAg, 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-FVIIIRAg antibodies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No of cases</th>
<th>Actin</th>
<th>FVIIIRAg</th>
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<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>++++, ++++, ++++, ++++</td>
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<tr>
<td>MDS</td>
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<td>+, ++++</td>
<td>+, ++++, ++++, +</td>
</tr>
</tbody>
</table>

+++ = 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.
References


Boqué, Pujol-Moix, Linde, Murcia, Guanyabens, Soler


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