Editorial

Importance of immunohistochemistry in the diagnosis of skin tumours

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Immunohistochemistry (IHC) refers to the process of localizing proteins in cells of a tissue section, using the principle of antigen-antibody binding. It takes its name from "immuno," in reference to antibodies used in the procedure, and "histo," meaning tissue. Currently this technique is widely used in the diagnosis and treatment of almost all sorts of tumours.

Visualising an antigen-antibody-interaction can be accomplished in a number of ways. In common practice, an antibody is conjugated to an enzyme, such as peroxidase, that catalyses a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, such as FITC, rhodamine, or Texas Red. The latter method is of great use in confocal laser scanning microscopy, which is highly sensitive and can also be used to visualise the interactions between multiple proteins. The later technique, however, is not commonly used.

The antibodies used in IHC can be polyclonal or monoclonal. Monoclonal antibodies are generally considered to exhibit greater specificity while the polyclonal antibodies are heterogeneous mix of antibodies that recognize several epitopes. Antibodies can also be classified as primary or secondary. Primary antibodies are raised against an antigen of interest and are typically unconjugated (unlabelled), while secondary antibodies are raised against the primary antibodies and are usually labelled with either biotin or a reporter enzyme such as alkaline phosphatase or peroxidase.

In the procedure, thin (about 4-40 µm) slices are taken of the tissue of interest, or if the tissue is not very thick and is penetrable, it is used whole. The slicing is usually accomplished by the use of a microtome, and slices are mounted on slides. Two types of techniques are used for the immunohistochemical detection of antigens in tissue, the *direct* method and the *indirect* method. In both the methods, the tissue is first treated to rupture the membranes, usually by using a kind of detergent called Triton X-100. The *direct method* is a one-step staining method, and involves a labelled antibody reacting directly with the antigen in tissue sections (**Figure 1**). This technique utilizes only one antibody and the procedure is therefore simple and rapid. However, this is not a sensitive method of detection of antigens as there is very little signal amplification with this procedure and is therefore less commonly used than indirect methods.

The *indirect method* of immunohistochemical staining involves an unlabelled primary antibody (first layer),

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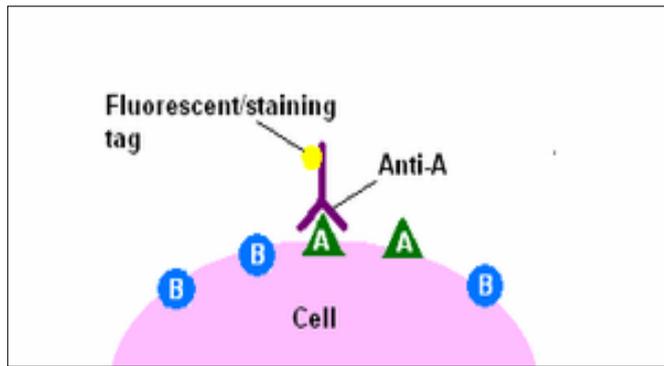Tel No. 061-5316154
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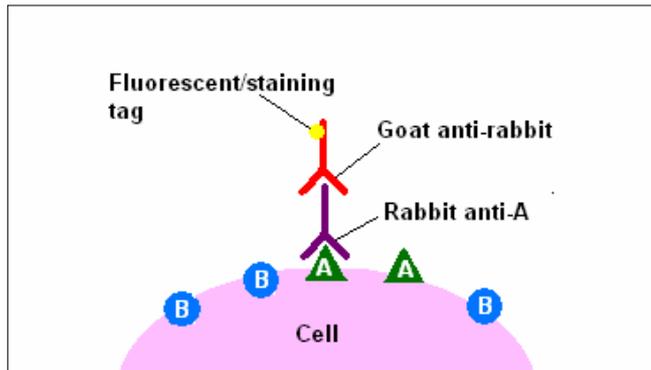**Figure 1** Technique of direct immunohistochemistry.

Figure 2 Technique of indirect immunohistochemistry.

which reacts with tissue antigen, and a labelled secondary antibody (second layer) which reacts with the primary antibody (**Figure 2**). This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody.

In common practice, secondary antibody is coupled with peroxidase. This reacts with 3,3'-diaminobenzidine (DAB) to produce brown staining (a process known as DAB staining). Hence the positive staining is judged by the intensity of the brown pigmentation of the specimen. The indirect method, aside from its greater sensitivity, also has the advantage that relatively a small amount of standard conjugated (labelled) secondary antibodies are required to be generated.

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. Following are a few applications of this technique in the diagnosis of skin tumours.

1. Squamous cell carcinoma (SCC) stains with epithelial membrane antigen (EMA) while basal cell carcinoma does not.[1]

2. On the contrary, basal cell carcinoma (BCC) stains very strongly with BerEP4 and bcl-2, this is negative in SCC and other trichoblastic tumours that mimic basal cell carcinoma on H&E staining.[2]

3. Trichoepitheliomas and trichoblastomas are characterized by the presence of papillary mesenchymal bodies (stain with CD10) and Merkel cells (CK20, Cam 5.2, EMA).[3]

4. Malignant melanomas stain strongly with S-100 (very sensitive but not so specific) and Milan-A (very specific but not so sensitive). Overexpression of p-53 correlates with invasive and metastasis melanoma.[4]

5. In atypical spindle cell tumours, immune markers help in differentiating between leiomyosarcomas (smooth muscle actin, desmin), SCC (EMA, pankeratin), melanoma (S-100 and Melan-A), atypical fibroxanthoma (CD10) and dermatofibrosarcoma protuberans (CD34).[1]

6. Histiocytic tumours are stained with S-100 and CD-68. Langerhans cells in addition to above two markers stain very strongly with C1a (diagnostic).[5]

7. Mast cells stain with CD117.[6]

8. In mycosis fungoides the lymphocytes are CD3, CD5 and CD7 positive. The increase in CD4/CD8 ratio to 4-5:1 and loss of either CD 5 or 7 staining is very diagnostic.[7]

9. For pleomorphic tumour, staining with S-100, pankeratin and leucocyte common antigens usually determine the origin of the cells.[1]

There is a never ending list of immunostains which have made life of dermatopathologists very easy as now they can diagnose the tumours very accurately and help the dermatologists in predicting the prognosis. Hence the knowledge of immunohistochemistry is almost mandatory for dermatologists in general and dermatopathologists in particular.

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