Immunohistochemistry

Immunohistochemistry (IHC) or immunocytochemistry is a method of localizing specific antigen in tissue or cells based on antigen antibody reaction. IHC is the way of validating morphological findings. It helps in tumor diagnosis and classification, identify prognostic and predictive markers.

IHC has a long history that dates back more than 70 years, when Coons1 first developed immunofluroscence technique to detect corresponding antigen in frozen tissue section. At oxford, Taylor and Burns2 developed the first successful demonstration of antigens in routinely processed formalin fixed paraffin-embedded sections. Since the early 1990s, IHC has been applied in routine formalin fixed paraffin embedded tissue. 3-4

Validation of reagents, protocols, controls and staining results are vital steps of IHC. The basic principles and protocols for fresh-frozen tissue sections are the same as those for paraffin sections, except that the antigen retrieval and dewaxing procedures are not required for frozen tissue sections. Titrations may also differ and must be separately optimized.

Basic principles:

Antigen-antibody recognition is based on the three-dimensional (3D) structure of protein or some other antigen, which may be compromised by formalin-induced modification of protein conformation ("masking") but is restored in part by Antigen retrieval.

Anti-A antibody binds specifically to antigen A in the tissue section. Antigen B (B) is depicted as a second antigenic determinant that is part of the anti-A molecule; anti-B antibody, made in a second species, will bind to this determinant. Thus anti-B, the so-called secondary antibody, can be used to locate the site of binding of anti-A, the primary antibody, in a tissue section.

Basic IHC procedure

Antigen retrieval (AR)-Enzymatic digestion (proteinase or trypsin), heat treatment (Microwave, water bath or autoclave)

Blocking of non-specific background staining

Incubation with primary antibody in humidity chamber

Add avidin-biotin-peroxidase complex, which binds to secondary antibody

Add 3, 3' diaminobenzidine (DAB) as a chromagen (color changing reagent), with hematoxylin (Mayer's) counterstaining

Theranostic Application

IHC is becoming increasingly important for the evaluation of predictive markers that can help select patients who may respond to particular targeted therapies. Some of these CD117 for GI stromal tumors, Herceptin (Genentech, South San Francisco, CA) for HER2-positive breast cancers, and rituximab for CD20-positive lymphomas.

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