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Biological Note on Immunohistochemistry

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Commentary

A laboratory method that uses antibodies to check for certain antigens (markers) in a sample of tissue. The antibodies are usually linked to an enzyme or a fluorescent dye. After the antibodies bind to the antigen in the tissue sample, the enzyme or dye is activated, and the antigen can then be seen under a microscope. Immunohistochemistry is used to help diagnose diseases, such as cancer. It may also be used to help tell the difference between different types of cancer.

Immunohistochemistry (IHC) is the most common application of immunostaining. It involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. IHC takes its name from the roots "immuno", in reference to antibodies used in the procedure, and "histo", meaning tissue (compare to immunocytochemistry). Albert Coons conceptualized and first implemented the procedure in 1941.

Visualising an antibody-antigen interaction can be accomplished in a number of ways, mainly either of the following:

- Chromogenic immunohistochemistry (CIH), wherein an antibody is conjugated to an enzyme, such as peroxidase (the combination being termed immunoperoxidase), that can catalyse a colour-producing reaction.
- Immunofluorescence, where the antibody is tagged to a fluorophore, such as fluorescein or rhodamine.

Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis). Immunohistochemistry is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue.

Immunohistochemistry is a valuable tool for the identification and visualization of tissue antigens in biological research and clinical diagnostics. Immunohistochemistry can characterize various biological processes or pathologies, such as wound-healing, immune response, tissue rejection, and tissue–biomaterial interactions. Specific antigen–antibody reactions can localize key molecules (e.g., cytokines, enzymes, transcription factors) associated with each process within the tissues. Combined use of antibodies for such molecules and cell type-specific markers can identify the major cell sources and examine cell phenotypic changes (e.g., differentiation, activation). The careful validation and protocol optimization, as well as selection of the proper microscopy methods (bright-field vs. fluorescence microscopy), enable us to obtain specific and reproducible results. In addition, various newly developed fluorescent dyes, excellent color contrast, high resolution, and the possibility of simultaneous multicolor imaging make immunofluorescence microscopy a convenient tool for studying pathobiological processes at the cellular level. This chapter provides a general introduction to immunohistochemistry, including background information about antibody–antigen interaction, labeling techniques, tissue processing, basic immunohistochemical methods, and data interpretation. It also discusses the impact of immunohistochemistry in biomaterial research and current understanding of the pathological processes of wound-healing after implantation.

Preparation of the sample is critical to maintain cell morphology, tissue architecture and the antigenicity of target epitopes. This requires proper tissue collection, fixation and sectioning. A solution of formalin is often used to fix tissue, but other methods may be used.

Immunohistochemistry (IHC) is a method to identify specific antigens within tissue sections utilizing an antigen-specific antibody. Detection at the light microscopic level of antigen–antibody interactions can be achieved by labeling the antibody with a substance that can be visualized, either by conjugation to a fluorescent marker or enzyme followed by colorimetric detection. Immunologic detection of antigens dates to the early 20th century when Marrack demonstrated that anti-typhoid and anti-cholera sera-labeled with diazotized benzidine-azo-r-salt imparted a red color to the bacteria. Although ground breaking for immunological detection of antigens, Coons determined this labeling method to be relatively insensitive when applied to tissues and subsequently described assays utilizing fluorescent-labeled antibodies in fixed tissues, but interpretation was confounded by the enhanced endogenous fluorescent activity in formalin-fixed tissue.

In 1966, Nakane described a method of antigen detection in tissue using an antibody conjugated to an enzyme (horseradish peroxidase) and utilized a colorimetric substrate that could be detected by light microscopy, which is the theoretical basis of most modern tissue-based immunohistochemical assays. This chapter will focus on detection of rabies virus (RABV) antigens in Formalin-Fixed Paraffin-Embedded (FFPE) tissues. The materials and methods describe a single protocol. However, the reader is encouraged to investigate the many alternative non-proprietary and proprietary protocols that are also available. Detection of antigens in FFPE tissues presents a unique diagnostic challenge regarding validation of the assay and interpretation of the results. Readers unfamiliar with this method should seek input from technicians and pathologists experienced with assay design and interpretation, particularly related to variation in basic protocols as to the impact upon test sensitivity and specificity.

Since IHC involves specific antigen–antibody reactions, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes, and tissue structures. Therefore, IHC has become a crucial technique and is widely used in many medical research laboratories as well as clinical diagnostics.

Another important advantage of IHC is that it can also be used to detect organisms in cytological preparations such as fluids, sputum samples, and material obtained from fine needle aspiration procedures. This can be very helpful in certain situations such as detection of pneumocystis from the sputum of an immunocompromised patient who needs rapid and precise confirmation of infection in order to begin immediate and appropriate therapy.

IHC can also be used to determine the function of specific gene products in fundamental biological processes such as development and apoptosis. Using a custom made monoclonal antibody against p53 homologue of the pro-apoptotic pathways of p53 was identified.

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