Immunohistology: an introduction to selective cell labeling as an essential element in the study of structure-function relationships

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Life represents the animate part of the world within a variety of textures. For understanding life we have to analyse the biomolecules in context with the underlying structure as well as their role in practicing and maintaining structure-function relationships. Such studies cover phylogeny, ontogeny and the various pathologies. Finally, all identifications serve the challenge in deciphering of how the central functions of the cells are performed by their self-assembled “machines” and by their complex set of interactions between multiple molecular blocks.

In a simplified manner, several questions require answers as to how (a) structures are built; (b) life functions work; (c) pathologies develop; (d) repairs are done; and (e) last not least, the question to the original sense of ontogeny and phylogeny. To these efforts, sophisticated tools are necessary, and, together with traditional methods, a number of modern biochemical and histological concepts have an outstanding role.

Research and applications in life science rely to a large extent on molecular recognition patterns. To this end, techniques are in steady development and microscopes are of outstanding importance. The identification of organ structure is one aspect and recognition of the basic mechanisms is another aspect. The codes can be found in studies of the spatio-temporal relationship of molecular cell construction with their dynamic developments in diverse situations.

The progress of genomic technologies is a means in systematic analysis of samples at the molecular level. Although these techniques provide important insights into the living process, all the data regarding DNA sequence variations or RNA transcript levels of cells will only provide a partial picture. In many if not all disease cases proteomics are necessary to complete the picture. In this context, the availability of high-quality antibodies will facilitate studies application in proteomics and diagnostics. The need of biomarkers with high sensitivity and specificity are the key for diagnostics of complex diseases and for improved risk stratifications. Biomarkers are a class of biological molecules with characteristic features which can be measured and visualized. Their outstanding role in the identification of normal and diseased states is generally accepted. Biomarkers can give informations whether a disease is just occurring or in progression, and, are possibly parameters for diagnostics, prognosis and the assessment of sequential events.

A main topic of structure-function analysis is covered by microscopic imaging. In this context, molecular labelings with specific probes are predominant. Specific probes are f.e. antibodies as used in immunohistology. The basic concept of immunohistology is very simple in combining different disciplines such as immunology, histology and chemistry. The development of immunohistology has a long history. The fundamental ideas were laid by AH

COONS and co-workers in the 1940s with the labeling of antibodies by fluorochromes. Their original intent was the identification of putative foreign antigens in affected tissues in certain diseases. Soon, it became evident that this technique would be of great value for the detection of all types of antigens in cells.

In the mean time, the methodology of immunohistology has become very complex with goals of high sensitivity and specificity as well as to detect multiple antigens simultaneously in a given biological specimen. Moreover, advances were made with so-called antigen retrieval methods to detect an exponentially rising number of antigens in routinely fixed tissues. Furthermore, microscopes, illumination equipment and electronic imaging devices have reached a level which enable morphological studies in an hitherto unexpected dimension.

Modern histology continues the traditions of the past by the integration of technical and scientific advances reflecting developments (a) in the 19th century such as the light microscope and histochemical stains; (b) in the 20th century such as electron microscopy, fluorescence microscopy and immunhistochemistry; and (c) in the 21th century marked by molecular and nanoscale techniques. We have to recognize that the success of immunohistology depended essentially on the following directions:

- The construction of *perfect microscope lenses*, based on ABBE's fundamental works on optics (diffraction limit of light) which are the basis for all mathematically calculated and manufactured lenses.
- The techniques of modern *microscope illumination, light filtering* and *imaging.*
- The entrance of specific *histological stains* characterized by a long research with natural and synthetic (aniline) dyes, enzyme chemistry, physicochemical dye stainings, and, finally the invention of *immunofluorescence* with all the subsequently developed immunostainings including molecular ligand techniques derived thereof.

We try to point out the complexity of this research field, i.e. immunohistology, by division of the whole subject into several chapters, each covering relevant arcticles dealing with methodology and applications. However, before we start with details and descriptions of immunohistological work, we'll have a look into the development of microscopes; by doing so, we pay tribute to those men who have brought the microscope to science.

Then, theoretical and practical bases of reagent preparation are given and followed by aspects in tissue sampling; general considerations are added by practical methods. The reader will also find a number of key references including citations of historical relevance. Finally, the use of many methods is outlined by experiments with biological models which are under our focus.

Selected publications for further readings

Coons AH *et al*. (1941)
Coons AH *et al*. (1942)
Coons AH and Kaplan MH (1950)
Coons AH *et al*. (1951)
Coons AH (1954)
Coons AH (1958)
Singer SJ (1959)
Singer SJ and Schick AF (1961)
Avrameas S (1970)

Coons AH (1971)
Nairn RC (1976)
Sternberger LA (1979)
Kuhlmann WD (1984)
Hell SW and Stelzer EHK (1992)
Chalfie M *et al*. (1994)
Masters BR (2006)
Stewart CN (2006)

Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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