Histochemistry as a versatile research toolkit in biological research, not only an applied discipline in pathology

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Abstract

The impressive progress of histochemistry over the last 50 years has led to setting up specific and sensitive techniques to describe dynamic events, through the detection of specific molecules in the very place where they exist in live cells. The scientific field where histochemistry has most largely been applied is histopathology, with the aim to identify disease-specific molecular markers or to elucidate the etiopathological mechanisms. Numerous authors did however apply histochemistry to a variety of other research fields; their interests range from the microanatomy of animal and plant organisms to the cellular mechanisms of life. This is especially apparent browsing the contents of the histochemical journals where the articles on subjects other than pathology are the majority; these journals still keep a pivotal role in the field of cell and tissue biology, while being a forum for a diverse range of biologists whose scientific interests expand the research horizon of histochemistry to ever novel subjects. Thus, histochemistry can always receive inspiring stimuli toward a continuous methodological refinement.

Introduction

In the Merriam-Webster dictionary (https://www.merriam-webster.com/dictionary/histochemistry), Histochemistry is defined as "a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues", while in the English Oxford Dictionary (https://en.oxforddictionaries.com/definition/histochemistry) it is "the branch of science concerned with the identification and distribution of the chemical constituents of tissues by means of stains, indicators, and microscopy". These definitions seem to assert that histochemistry has the main function to statically describe the chemical composition of tissues and cells, in the framework of their structural organization.

Actually, since the end of the 19th Century and for some decades, histochemistry was a discipline were new dyes and staining methods were seen as a means to discriminate morphology through the light microscope, irrespective of their capability to detect specific chemicals at the tissue or cellular level.[1] However, already in the first half of the 20th century the classical textbooks on histochemistry by Lucien Lison[2] and David Glick[3] focused on the chemistry of cells and tissues, in the attempt to improve the knowledge on cell biochemistry through the application of chemically specific reactions on sample sections. Based on this tenet, in 1958 A.G. Everson Pearse[4] wrote that "Histochemistry is concerned particularly in the correlation of structure with function", and cleverly foresaw that "if advances are to be made in our knowledge of the mechanisms of pathological processes then new methods must be invented which will describe the organization of cellular events and which will be sensitive enough to detect early functional changes".

Actually, the impressive, still ongoing progress of histochemistry over the last 50 years has led to setting up specific and sensitive techniques suitable for describing dynamic events, with attention to the detection of specific molecules in the very place where they exist in live cells.[5-7]

In the attempt to ever more precisely describe the "chemistry" of a diseased tissue, refined techniques to detect single molecular species are routinely used on sections, generally from fixed and embedded samples.[8] *In situ* hybridization and especially immunohistochemistry are widely applied to identify and localize specific nucleic acid sequences or proteins, and multiple techniques have been developed to simultaneously detect several different molecules on the same section. The more traditional multicolor immunofluorescence approach has in recent years been paralleled by mass cytometry where antibodies tagged with unique rare-earth-metal isotopes of defined atomic mass allow to localize up to more than 30 different proteins in a single tissue section.[9] As a complementary technique to the conventional histological examination and immunohistochemistry, enzyme histochemistry on cryosections effectively links biochemistry with morphology through the detection of an ongoing enzyme activity in its topographical localization: this makes it possible to obtain a metabolic mapping of tissues so that cell metabolic changes may be noticed as a consequence of pathological events or experimental treatments, even in the absence of apparent changes in the histological or immunohistochemical features.[10-12]

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Applications of histochemistry in the recent scientific literature

It was an obvious consequence that the scientific field where histochemistry has most largely been applied was histopathology, with the scope of identifying disease-specific structural indicators, or of elucidating the etiopathological mechanisms.[13] This also occurs nowadays.

In fact, browsing the scientific literature during the last ten years (source: the Scopus database, https://www.scopus.com/), it is easy to realize that the great majority (about 80%) of the published *histochemical* articles dealt with applications in human pathology (Figure 1). Describing the molecular organization of pathological tissues proved to be crucial to precisely diagnose a disease, and often to select the most appropriate therapeutic approach; furthermore, the microscopical observation of the spatial cellular organization and possible heterogeneity of tissue is especially important in cancer where different cell subpopulations may coexists, and their location in the tumor microenvironment may provide indication on the actual condition and the progression of the disease.[14]

On the contrary, during the same timespan, about 20% only were the published papers where histochemistry was explicitly reported as the main approach for investigating biological subjects other than human pathology (such as *e.g.*, cell biology, zoology, animal and human anatomy, botany, developmental biology, *etc.*), despite the wide range of investigated topics.

There is no reason to conclude that histochemistry has limited application outside pathology; more likely, histochemical techniques are used, sometimes unconsciously, by scientists who see histochemistry as an ancillary discipline even worthless to be

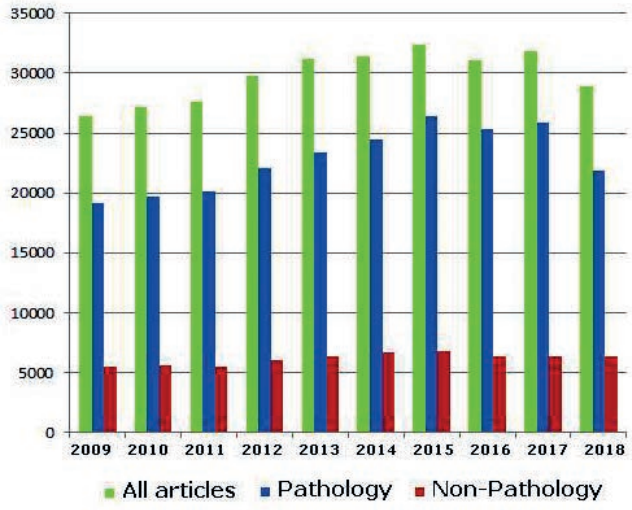

Figure 1. Number of scientific articles where histochemistry was used during the last ten years (source: https://www.scopus.com/). Most of the published papers (70 to 80%) were on pathology subjects.

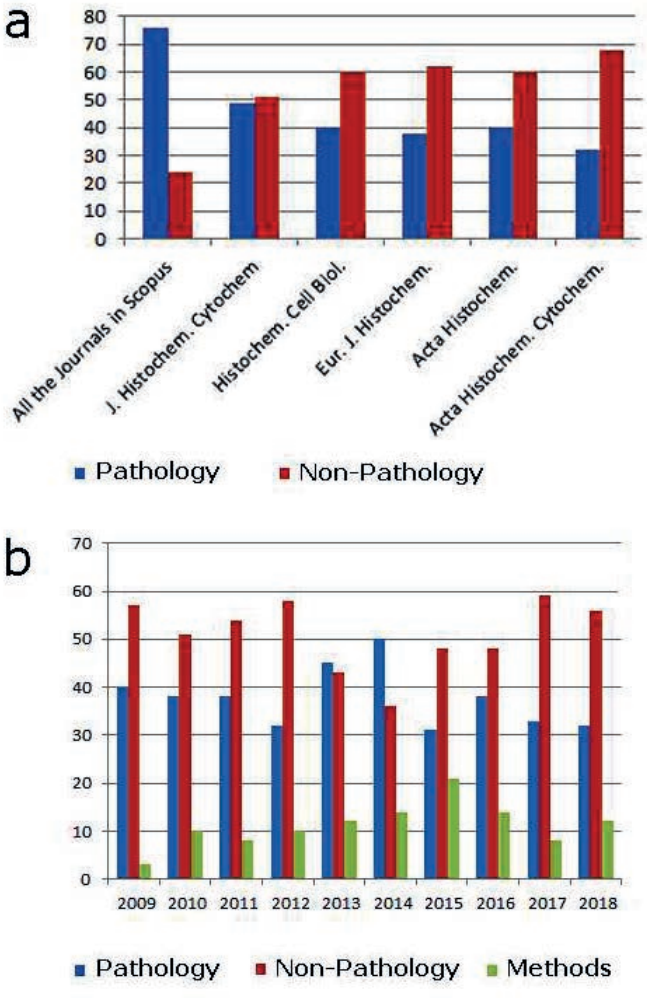

Figure 2. a) Mean percentage values of the histochemical articles published on pathology or non-pathology subjects in all the scientific journals indexed in the Scopus database or in some histochemical journals, during the last ten years. b) Percentage of scientific articles on pathology or non-pathology subjects, or on methods published in the European Journal of Histochemistry from 2009 to present.

mentioned. Perhaps, as Raymond Coleman wrote, "histochemistry is often mistakenly perceived as an archaic discipline, and its contributions to cell and molecular biology are not always given the credit it deserves".[15]

Interestingly, if we focus on strictly histochemical journals, the percentage of articles on *non-pathology* topics is much higher, from 50 to 70% of the published papers in the journals considered in Figure 2a.

Taking as an example the European Journal of Histochemistry, we may observe that the articles on human tumor or non-tumor diseases or on experimental medicine were, as a mean value, less than 40%, whereas especially in the last couple of years, the papers published on other biological areas did approach 70% (Figure 2b).

This evidence suggests that indeed the attention is still high for the histochemical detection of markers for tumor[16-27] or non-tumor[28-32] diseases, or for the use of histochemical methods to investigate the biological bases of a disease through approaches of experimental medicine.[33-36] Numerous are, however, the authors who applied histochemistry to many other research fields.

Basic biological processes such as DNA damage and repair, cell migration and cytoskeletal organization, or epithelial-mesenchymal transition were effectively described through specific histochemical reactions using experimental models *in vitro* or *in vivo*.[37-42] The biology of stem cells was extensively investigated through the labelling by specific molecular markers, during development and in the adult.[43-46] The molecular organization of cells and tissues was carefully analyzed[47-63] especially in poorly described species of mammals[55-57] and non-mammalian vertebrates[58-61] or in invertebrates,[62,63] while immunohistochemistry was crucial to elucidate the topographic distribution of cell lineages in different organs,[64-66] especially in the nervous system,[67-71] and during embryogenesis and the pre- or postnatal development,[72-79] or to evaluate the effects of environmental or pharmacological stress conditions.[80-85]

To expand the application potential of the histochemical approach and to increase the informational capacity of the histochemical evidence, novel techniques were set up[86-95] or original applications of established histochemical methods were described, using tissue and cell models from a variety of organisms (invertebrates, vertebrates or plants).[96-104]

Concluding remarks

Similar indications can be obtained examining the subjects of the articles pub-

lished in other histochemical journals (see *e.g.*[105-111]), and this attitude should especially be taken into account by scientists in basic biology whose interests range from the microanatomy of organisms to the cellular mechanisms of life, and who often use histochemistry and microscopy in their researches: sometimes, the results of their investigations can hardly be considered for publication in strictly discipline-oriented journals, whereas the histochemical journals may be the ones where their articles may fittingly be published to be read by a naturally open-minded and scientifically diversified readership.

Thus, histochemical Journals would keep their pivotal role in the field of cell and tissue biology, while becoming -even more than in the past- a forum for a diverse range of biologists. At the same time, expanding the research horizon to ever novel subjects would be vital to give histochemistry driving stimuli toward an unceasing methodological refinement.[112]

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