Histochemistry in biology and medicine: a message from the citing journals

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Abstract

Especially in recent years, biomedical research has taken advantage of the progress in several disciplines, among which microscopy and histochemistry. To assess the influence of histochemistry in the biomedical field, the articles published during the period 2011-2015 have been selected from different databases and grouped by subject categories. As expected, biological and biomedical studies where histochemistry has been used as a major experimental approach include a wide range of basic and applied researches on both humans and other animal or plant organisms. To better understand the impact of histochemical publications onto the different biological and medical disciplines, it was useful to look at the journals where the articles published in a multidisciplinary journal of histochemistry have been cited: it was observed that, in the five-years period considered, 20% only of the citations were in histochemical periodicals, the remaining ones being in journals of Cell & Tissue biology, general and experimental Medicine, Oncology, Biochemistry & Molecular biology, Neurobiology, Anatomy & Morphology, Pharmacology & Toxicology, Reproductive biology, Veterinary sciences, Physiology, Endocrinology, Tissue engineering & Biomaterials, as well as in multidisciplinary journals. It is easy to foresee that also in the future the histochemical journals will be an attended forum for basic and applied scientists in the biomedical field. It will be crucial that these journals be open to an audience as varied as possible, publishing articles on the application of refined techniques to very different experimental models: this will stimulate non-histochemist scientists to approach histochemistry whose application horizon could expand to novel and possibly exclusive subjects.

Introduction

The wideness of interest of a scientific discipline, and its impact on knowledge and the research progress may be estimated through the number of journals issued in its field, the quantity of articles published in a given time-span, and the number of citations these articles obtained. No doubt, this is a rough estimate but it may allow to quantitative and compare the data taken from qualified databases, and to foresee future developments.

During the last fifteen years, the number of journals published in the biomedical field has significantly increased: for instance, based on the Thomson Reuters’ Journal Citation Report (https://jcr.incites.thomsonreuters.com) the journals in the subject categories of Biology increased from 62 to 85, in Medicine (Experimental & Research) from 74 to 123, and in Oncology from 103 to 211, with an impressive (though hardly computable) surge of published articles.

Research in biomedical has taken advantage of the progress in several disciplines, first of all in molecular biology and related omics. Microscopy and histochemistry also played a major role, especially in recent years, by providing new instrumentations and refined techniques.1-4 In the attempt to assess the impact of histochemistry in the biomedical field, it may be interesting to look at the articles recently published in qualified journals, reporting the results of investigations performed by histochemical methods.

Main subjects of the histochemical articles published from 2011 to 2015

Browsing the records of PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Web of Science (http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discover/web-of-science.html) and Scopus (http://www.scopus.com/), the articles published in qualified journals where histochemistry or immunohistochemistry are present in the title/abstract or as key-words were about 87,000, 130,000 and 132,000 respectively, in the last five years (the numerical differences are essentially due to the different journals which are indexed: more medicine-oriented in PubMed, and more widely biology & medicine-oriented in Web of Science and Scopus).

By grouping these publications in subject categories, according to their contents (Figure 1), it is apparent that more than 40% deal with medical subjects (Pathology & Experimental Medicine); articles on Differentiation & Stem cells or Neurons & Muscle (more than 15% and 10%, respectively), as well as those on Methods (about 10%) are also present at high percentages. About 5% are those on Animal tissue biology and Hard tissue & Matrix, and only few on Tissue engineering or on Plant tissue biology.

Thus, in these five recent years, biomedical studies where histochemistry has been used as a major experimental approach included a wide variety of subjects, from basic to applied research on both humans and other animal or plant organisms.

The last five-years record of a multidisciplinary journal of histochemistry

Among the purely histochemical journals, the European Journal of Histochemistry - EJH has traditionally been devoted to publish papers on functional cell and tissue biology in animals and plants, cell differentiation and development, cell-to-cell interaction and molecular trafficking, nerve and muscle cell biology, with special attention to the cellular basis of diseases.

It is worth observing (Figure 1) that some differences exist in the distribution of subjects of the papers published in the European Journal of Histochemistry during the same five-years period, compared to the overall histochemical articles in the literature. Consistent with the latter ones, the majority (about 35%) of the published articles were on Pathology & Experimental Medicine, and the percentages found for Hard tissues & Matrix, Neurons & Muscle and Plant tissue biology were also similar as those for the whole histochemical production. On the contrary, the percentage of papers published on Animal tissue biology, on Methods and on Tissue engineer-
ing was higher in the *European Journal of Histochemistry*, whereas the one on Differentiation & Stem cells was lower.

Looking at the distribution of articles on these subjects during the last five years (Figure 2) may help to understand how the interests of this journal's authors changed over time, particularly in the most recent time. The articles in Pathology & Experimental Medicine ranged from more than 40% to about 20%, having been very numerous in 2013,5-15 while progressively decreasing in 201416-29 and 2015;30-40. Most of these papers were focused on the detection of tumor markers,7,10,13,26,29,33 but several were aimed at elucidating the mechanisms of carcinogenesis and tumor progression.2,23,26,29,33 There was steady interest for developmental investigations and stem cell biology with about 15% of the published articles in the last couple of years: molecular markers of cell differentiation were used to describe the process of placenta formation11,12,14,16,21,23,28,34 and organogenesis42-46 and to phenotype stem cells.47,48

Extracellular matrix and hard tissues have become increasingly important topics, demonstrating that histochemistry may be suitably applied in particular to investigate cartilage or dentin during the histogenetic process49-51 and in pathological conditions.52,53 Some papers were devoted to the temporomandibular joint in healthy and diseased subjects.54-59 While other proposed in vitro models for chondrogenesis50-62 or dentinogenesis.53 Techniques for culture in vitro were also used for tissue engineering, using different supports for growing and differentiating various cell types.54-67 Histochemistry thus proved to be an effective mean to characterize the structural and functional features of cells to be used for reconstructive medicine.

The incidence of articles on Neurons & Muscle has progressively decreased from 2013 to present, although a series of interesting article was published especially for muscle cells.68-81 In particular, it was observed that both the ultrastructural organization and the nuclear function of myonuclei and satellite cells' nuclei in the skeletal muscle from patients with myotonic dystrophy were similar to those in the muscles from sarcopenic individuals, suggesting that similar nuclear mechanisms may lead to skeletal muscle wasting.76,79

There was a steady and progressive rise in the percentage of papers presenting new methods or improved techniques: they were about 12% of the published articles in 201382-86 growing to more than 20% in 2014 and 2015.87-102 Actually, this is consistent with the scope of the *European Journal of Histochemistry*, which has always been an open forum for scientists to present new methods and techniques, and...
to discuss their experimental results, as it recently occurred for the molecules responsible for some characteristics of cell autofluorescence.\(^{93,103-105}\) In the attempt to localize specific molecules at high resolution, ultrastructural histochemistry proved to be especially suitable, and demonstrated by the several papers published.\(^{106-108}\) In particular, diaminobenzidine photo-oxidation was effectively used to visualize the endocytotic pathways after staining the plasma membrane with a fluorescent dye,\(^ {109}\) and to simultaneously detect immunogold-labeled antigens at the high resolution of transmission electron microscopy.\(^ {110}\)

As a journal of functional cytology, the European Journal of Histochemistry has traditionally published many papers on cell and tissue biology in a variety of Vertebrate and Invertebrate species. After a relative decrease in 2013 and 2014,\(^ {111,112}\) in the present year more than 20% of the published papers were on these subjects,\(^ {113-115}\) thus demonstrating that histochemical techniques are powerful tools for properly describing cell and tissue organization as well as functional microanatomy in different taxa of still poorly described organisms. In a comparative perspective, the histochemical evidence, in parallel with molecular data on protein and DNA, may help to elucidate the origin and evolution of cell and tissue physiology.

### Estimating the impact of the published articles through a survey of the citing journals

The overall influence of an academic journal on the scientific community is usually estimated by the impact factor, which is a measure reflecting the mean number of citations obtained by recent articles published in a given journal. This index does not take into account the subject category of the journals where the citing articles were published; but especially for journals devoted to a variety of subjects and techniques, as the European Journal of Histochemistry is, it should be interesting to consider this aspect, too.

The more than 200 articles published in the period 2011-2015 in this Journal have been cited in 340 different periodicals. Figure 3a shows the percentage of citations in these journal which have been grouped according to their main topic and their first subject category reported in the Thomson Reuters’ Journal Citation Reports.

As expected, the journals on histochemistry were those in which the articles published in the European Journal of Histochemistry were most frequently cited. In agreement with the evidence in Figure 1 and 2, a large fraction of the citations were in medical journals (dealing with both general and experimental medicine, and with oncology), and in journals on cell and tissue biology or on biochemistry and molecular biology.

In most of the articles published in the European Journal of Histochemistry, specific molecular complexes were detected, and their structural location in cells and tissues was related with function. In addition, several papers also described new methods or presented improvements of well-established techniques thus providing new opportunities for application to a wide variety of research subjects. It is therefore not surprising that citations are frequently found in journals of Neurobiology, Anatomy & Morphology, Pharmacology & Toxicology, Reproductive biology, Veterinary sciences, Physiology, Endocrinology, Tissue engineering & Biomaterials, Plant cell biology, as well as in multidisciplinary journals; significantly, the citations in histochemical journals of the articles published in 2011 and 2012 progressively decreased from 2013 to 2015, while increasing during the same timespan in the journal of medicine, pharmacology, physiology and tissue engineering (Figure 3b).

### Concluding remarks

In the last couple of decades, histochemistry has become a true molecular biology in situ, aimed at detecting single molecules in the very place where their functional role is exerted. The potential of histochemistry for diagnosis and prognosis has greatly increased by the use of refined techniques for investigating gene expression in situ. Vital histochemistry (chiefly, enzyme histochemistry)\(^ {116,117}\) provides evidence of the biochemical events driving and controlling cell physiology. The recent technological improvements in super-resolved light microscopy and in electron microscopy\(^ {118,119}\) as well as the progress in correlative microscopy,\(^ {120}\) and in imaging molecules in vivo\(^ {121,122}\) allowed to shed light on the molecular processes responsible for cell differentiation or which account for the onset a patholog-

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**Figure 3.** a) Percentage of citations in qualified journals of the articles published in the European Journal of Histochemistry in 2011-2015; the citing journals have been grouped according to their main topic and the first subject category in Thomson Reuters’ Journal Citation Report. b) Percentage of citations obtained from the different journals’ categories in the years 2013, 2014 and 2015 by the articles published in the European Journal of Histochemistry in 2011-2012.
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