Abstract

In agreement with the evolution of histochemistry over the last fifty years and thanks to the impressive advancements in microscopy sciences, the application of cytochemical techniques to light and electron microscopy is more and more addressed to elucidate the functional characteristics of cells and tissue under different physiological, pathological or experimental conditions. Simultaneously, the mere description of composition and morphological features has become increasingly sporadic in the histochemical literature. Since basic research on cell functional organization is essential for understanding the mechanisms responsible for major biological processes such as differentiation or growth control in normal and tumor tissues, histochemical Journals will continue to play a pivotal role in the field of cell and tissue biology in all its structural and functional aspects.

Histochemistry and Cytochemistry in recent literature

According to the web definitions, Histochemistry is the branch of science that deals with the chemical composition of the cells and tissues of the body or a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues (Free online Dictionary by Farlex: http://www.thefreedictionary.com). On the other hand, Cytochemistry is the study of intracellular distribution of chemicals, reaction sites, and enzymes, often by means of staining reactions, radioactive isotope uptake, selective metal distribution in electron microscopy, or other methods (Free Merriam-Webster Dictionary: http://www.merriam-webster.com/).

All these definitions seem to suggest that Histochemistry and Cytochemistry are to be considered as ancillary disciplines which may purely describe the chemical composition of cells and tissues through the application of a reasonably wide collection of methods and techniques. An even cursory survey of the articles published in the histochemical Journals during the last few years provides a much more exhaustive representation of the actual scope and potential of Histochemistry and Cytochemistry in the field of cell and tissue biology (as review articles, see for instance1-3). Indeed, the application of cytochemical techniques to light and electron microscopy has increasingly been addressed to elucidate the functional characteristics of cells and tissue under different physiological, pathological or experimental conditions; in fact, the mere description of composition and morphological features has become increasingly sporadic.

This is consistent with the evolution of histochemistry over the last fifty years: thanks to the impressive advancements in microscopy sciences, the current histochemical approach essentially aims to locate molecules in the very place where they exert their biological roles, and to dynamically describe specific chemical processes in living cells. This is apparent from a review of the articles recently issued on the European Journal of Histochemistry, an example of a publication which is explicitly devoted to functional cytology and histology.

Consistent with the large number of published articles in the literature on histochemical applications (more than 32,000 in 2011-2013, according to http://www.ncbi.nlm.nih.gov/pubmed), most of the papers concerned investigations on different pathologies, with special attention to tumor biology. The large majority of these papers focused on the molecular bases of diseases14-12 and on carcinogenesis,13-21 In particular, immunohistochemistry was used as a suitable tool for labeling diagnostic tumor markers22-27 (often in a multiple way) or prognosis-predicting indicators,28-29 and for detecting the expression of specific molecules in premalignant lesions.30

The distribution and activity of specific proteins was investigated in different animal or plant cells and tissues,31-35 and was often compared with the ectopic relocation of the same molecules under pathological conditions36,37 or after the application of experimental stimuli or therapeutic agents.40-51 The immunohistochemical detection of a given protein or the recognition of a specific enzyme activity was never aimed to purely describe cell features in a micro- (or ultramicro-) anatomical perspective. In fact, it clearly emerged that not only the presence of a given molecular species but also its proper subcellular location are essential for assuring cell and tissue normality.

The expression of specific protein marker was assessed during pre- and post-natal development in mammalian species,52-54 starting from the process of oocytes maturation and elimination.52,53-55 The development of heart and skeletal muscle was the subject of several papers, where special attention was paid to stem cell populations and their molecular characteristics.56,57,58 Here too, the unusual localization or translocation of specific proteins was considered as an important evidence,59 all the more so as it occurs under pathological conditions (e.g., in myotonic dystrophy or in sarcopenia, i.e., the loss of muscle mass and function physiologically occurring in the aging process).57,56-77 As demonstrated by ultrastructural immunocytochemistry, the dystrophic phenotype would depend on a general alteration in the post-transcriptional maturation of pre-mRNAs. Interestingly, the accumulation of pre-mRNA processing factors in the myonuclei of dystrophic patients is reminiscent of the nuclear alterations found in age-related sarcopenia. Consistently, it was observed that in vitro myoblasts derived from satellite cells of dystrophic patients show cell senescence features and alteration of the pre-mRNA processing pathways earlier than the myoblasts from healthy subjects.57 These results suggested possible common cellular mechanisms accounting for skeletal muscle wasting in myotonic dystrophy and sarcopenia.67,72

The effect of physical exercise on the structural features of skeletal muscle cells (both myocytes and satellite cells) has repeatedly been investigated,57,78 but so far much less attention has been paid to the consequence of exercise on the myotendineous junction:77,78 Molecular and ultrastructural analyses demonstrated that the myotendineous junction is able to adapt to increased tensile forces by enlarging the muscle-tendon contact area, thus increasing mechanical resistance.

Exercise training was found to have anti-inflammatory effects and promote atherosclerotic plaque stabilization in a mouse model of diabetic atherosclerosis,79 while continuous...
cyclic mechanical tension increases the expression of the ank gene (coding for a transporter of inorganic pyrophosphate from cells) in endplate chondrocytes through specific molecular pathways. This confirms that changes in ank expression may influence calcification in the intervertebral disc.

The importance of histochemistry for investigating the structural features and function of hard tissues is confirmed by several papers, where immunocytochemistry and RT-PCR or electron microscopy were often performed in an integrated approach. The dentin extracellular matrix proteins, DMP1 and DSP (which are produced by odontoblasts involved in dentin mineralization), have been studied in human sound versus sclerotic dentin: these proteins were more abundant in cartilaginous tissues, suggesting that odontoblasts are actively engaged in the biomineralization of dentin.

Human dental pulp cells were isolated and cultured in vitro, where reparative dentinogenesis was simulated by different odontogenic inducers. Odontoblast differentiation was monitored by histochemical staining procedures and electron microscopy in parallel with biochemical and molecular techniques. It was demonstrated that odontoblast markers (such as DMP1, DSP and type I collagen) were expressed in differentiated cells, and that DSP and DMP1 were differently located during odontoblast differentiation. The combined qualitative and quantitative assays used confirmed that this in vitro model can usefully elucidate the dynamic processes occurring in vivo during tooth repair.

It is worth noting that there was increasing interest for the application of histochemical techniques in regenerative and reparative medicine. An unexpected heterogeneity of adipocyte populations with different potential as sources for stem cells were described in the human trochanteric fat pad, and an interesting article was published on the quantitative assessment of lipid droplet accumulation in cultured adipogenic cells, as a parameter of adipocyte maturation. Either 3D scaffold-free fusion cultures or scaffoldings (either made on natural biomaterials or on synthetic polymers) were used in vitro and in vivo to enhance chondrogenesis. There, immunohistochemistry was instrumental in monitoring proliferation and differentiation of chondrogenic cells through the changes in the expression of specific proteins.

The potential of immuno-histochemistry for elucidating nerve cell differentiation during development was apparent in the review article by Oboti et al. on the rodent olfactory system as well as in other papers concerning the nervous system in mammalian species and in invertebrates.

Due to the heterogeneity of the animal models, tissues and experimental conditions, analytical methods need to be continuously adjusted and refined, as demonstrated by the number of technical notes which have recently been published in the European Journal of Histochemistry.

An appropriate fixation is the essential precondition for preserving an adequate morphology and performing a correct histochemical analysis: comparing the effects of different procedures on particular cells is therefore important, and fixatives alternative to the traditional ones may be proposed to make the application of diagnostic techniques easier.

The optimization of enzyme-histochemical techniques or a triple immunoenzymatic method employing primary antibodies from same species and same immunoglobulin subclass have been suggested, while new methods have been proposed for the quantitative evaluation of fluorescence signals and for imaging dynamic processes in vitro or in vivo.

Diaminobenzidine photo-oxidation has successfully been applied for tracking the intracellular location of fluorescently labeled nanoparticles at transmission electron microscopy. Photo-oxidation procedures proved to be suitable also for the simultaneous ultrastructural analysis in the same sample, and for gold immunolabelling in cultured cells, thus allowing to co-localise the photoconversion product and a variety of antigens at the high resolution of electron microscopy.

Concluding remarks

With the aim of understanding the mechanisms responsible for major biological processes such as differentiation or growth control in normal and tumor tissues, basic research on cell functional organization is fundamental.

Progress in microscopy techniques and the existing wide variety of refined histochemical methods provide unique opportunities for approaching biological processes in living cells by a true molecular biology in situ.

This confirms that histochemical journals maintain their pivotal role in the field of cell and tissue biology in all its structural and functional aspects, and that they should become the means to present original findings or proposing methodological and technological improvements, as well as to openly discuss founding ideas and theories even more than in the past.

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