Identifying pathological biomarkers: histochemistry still ranks high in the *omics* era

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Abstract

In recent years, *omic* analyses have been proposed as possible approaches to diagnosis, in particular for tumours, as they should be able to provide quantitative tools to detect and measure abnormalities in gene and protein expression, through the evaluation of transcription and translation products in the abnormal *vs* normal tissues. Unfortunately, this approach proved to be much less powerful than expected, due to both intrinsic technical limits and the nature itself of the pathological tissues to be investigated, the heterogeneity deriving from polyclonality and tissue phenotype variability between patients being a major limiting factor in the search for unique *omic* biomarkers. Especially in the last few years, the application of refined techniques for investigating gene expression *in situ* has greatly increased the diagnostic/prognostic potential of histochemistry, while the progress in light microscopy technology and in the methods for imaging molecules *in vivo* have provided valuable tools for elucidating the molecular events and the basic mechanisms leading to a pathological condition. Histochemical techniques thus remain irreplaceable in pathologist's armamentarium, and it may be expected that even in the future histochemistry will keep a leading position among the methodological approaches for clinical pathology.

Introduction

Identification of reliable and unequivocal diagnostic and prognostic markers is the ultimate goal of pathologists. Following the pioneering intuition of Giovanni Battista Morgagni (1682-1771), who first attempted to relate changes in macroscopic anatomy to the altered physiological features characterizing diseases, pathologists aim at detecting the most appropriate biomarker(s) enabling recognition of the earliest signs of a pathological condition.

In recent years, genomics and, especially, proteomics and transcriptomics did enter the pathologist's practice, and were initially foreseen as the final approach to diagnosis, in particular for tumours. In principle, *omic* analyses should provide quantitative tools to detect and measure abnormalities in gene and protein expression, through the evaluation of transcription and translation products in the abnormal *vs* normal tissues.[1]

Unfortunately, this approach proved to be much less powerful than expected, due to both intrinsic technical limits and the nature itself of the pathological tissues to be investigated. In fact, results from *omic* analyses reflect the contents in transcripts or proteins of whole cell extracts, whereas the occurrence of gene expression abnormalities in a pathological specimen may often be restricted to minor tissue fractions: this makes almost impossible to detect tiny quantitative changes in one or few aberrantly expressed molecules. The heterogeneity deriving from polyclonality and tissue phenotype variability between patients is another major limiting factor in the search for unique *omic* biomarkers. As a consequence, it has been suggested to use complex bioinformatic analyses to effectively mine heterogeneous quantitative data sets and extract their qualitative contents;[1] a consensus, however, has not yet been reached on how to deal with *omic* data in the attempt to identify suitable pathological biomarker(s).

The present role of histochemistry in clinical pathology

It is since the 1950's that histochemistry has been recognized as the discipline linking morphology (microanatomy and histology) and biochemistry, and has provided pathologists with a versatile armamentarium of methods and procedures enabling the recognition *in situ* of significant pathological markers.[2,3]

The advantage provided by the histochemical approach is the possibility to detect *in situ*, by microscopy techniques, changes in cell phenotype taking place even in limited portions of a pathological tissue: thus quantitative changes either in the percentage of cells positive for a given marker or in the intensity of the final reaction products within the cells allow reliable diagnostic conclusion through the comparison with the normal tissue, and well experienced pathologists often improve differential diagnosis by selecting a panel of appropriate histochemical markers.

On 12-15 October 2011, the 53rd Symposium of the Society for Histochemistry has been held in Munich (Germany) with the significant subtitle *Current role of Histochemistry in Preclinical and Clinical Research* (http://www.helmholtz-muenchen.de/histochemistry2011/home/index.html). Also during the 34th National Congress of the Italian Society of Histochemistry,[4] more than one third of the scientific contributions concerned the application of histochemical techniques to pathological models, in humans and other Vertebrates.

This interest for the application of histochemistry in the pathological field is confirmed in the international literature: during the last two years, out of the about 25,000 articles dealing with the application of histochemical techniques published in peer-reviewed journals, more than 50% concerned pathological (mostly tumour) subjects (source: http://www.ncbi.nlm.nih.gov/pubmed/).

Consistently, the percentage of published articles on pathological topics ranged between 25% and 40%, also in the most traditional journals on histochemistry, i.e. *Histochemistry and Cell Biology* (http://www.springer.com/medicine/anatomie/journal/418), the *Journal of Histochemistry and Cytochemistry* (http://jhc.sagepub.com/), and the *European Journal of Histochemistry* (www.ejh.it).

A review of the contents of the articles appeared in this latter journal showed that the majority of them were devoted to tumours.[5-24] As expected, immunohistochemistry has been mostly applied, and the differential expression of proteins in diseased *vs* normal tissues has often been investigated by a multiparametric approach.[7,11,12,14,15,22] A few papers were focussed on the unusual expression of proteins demonstrating possible heterogeneity within tumours classified in a single type,[6,17] while proliferation, differentiation and apoptotic markers have been used to elucidate differences in the progression of malignancy and

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in the metastatic potential of tumour cells *in vivo* and *in vitro*.[6,8,9,14,16,19-21,23,24]

Muscle wasting, due to physiological sarcopenia or dystrophy, has been widely considered:[25-30] the simultaneous application of histochemical techniques at light and electron microscopy allowed to relate the changes in cellular phenotype with altered gene expression. Changes in the structural and functional features of the cell nucleus of myocytes and satellite cells proved to be especially suitable to detect alterations not only in the organization of muscle fibres, but also in the regeneration potential of skeletal muscle under pathological conditions and aging.[25-28] Skeletal muscle has also been the subject of three interesting technical reports;[30-32] and other notes on techniques have been published concerning the preparation of pathological samples to be observed in bright field or fluorescence microscopy.[33-36]

Histological, histochemical and morphometrical techniques have been used to investigate the effects of intoxication, infection or vaccine administration on several organs and tissues from different Vertebrate species, from fish to mammals:[37-43] here too, the potential of light and electron microscopy and histochemistry has been exploited to identify minute changes in the structural organization and function of cells which heterogeneously take place in different tissues.

A growing interest is apparent for mineralized tissues, with special attention to joint alterations during development and under pathological conditions:[44-49] in particular, articular disks have been the subject of several papers, as the changes in their composition and organization are indicative features to understand pathological processes of internal derangement.[45-49]

In the wide variety of pathological conditions that have been considered in the published articles, histochemical techniques have been used not only to detect specific pathological markers,[50,51] but also to evaluate the effect of therapies[52] and to investigate the role of specific signalling molecules in the onset of pathological phenotypes.[53]

Oxidative stress is known as a powerful factor inducing pathological processes in cells by modifying their redox state: alterations in some enzyme activities have been investigated with special attention to those occurring as a consequence of hypoxia-reoxygenation.[54-57] The evidence that hypoxia and aging in the myocardial tissue are controlled by p53 and telomerase activity[57] confirms that molecular events modifying nuclear structure and function often play a pivotal role in pathogenesis; this is apparently confirmed by several articles in which nuclear histochemistry has been profitably used to elucidate basic mechanisms leading to the pathological cell phenotype.[23-27,58-60]

Finally, a new frontier of histochemistry seems to open in the field of tissue engineering,[61,62] transplantation,[63] and adipocyte-derived tissue reconstruction.[64,65]

Conclusions

The application of refined techniques for investigating gene expression *in situ* has greatly increased the diagnostic/prognostic potential of histochemistry, while the progress in light microscopy technology and in the methods for imaging molecules *in vivo* has provided a valuable tools for elucidating the molecular events and the basic mechanisms leading to a pathological condition.

In particular, nowadays two techniques are being utilized on pathological material, and they start from opposite directions: the already established super-resolution techniques such as STED (Stimulated Emission Depletion) microscopy[66] are now being tested on cells and subcellular structures, for purely diagnostic purposes, while MALDI (Matrix Assisted Laser Desorption/Ionization) analysis[67] only needs to approach a sufficiently high resolution to be profitably used at the cellular level. Consistently, in his closing statement to the 53rd Symposium of the Society for Histochemistry, the President of the Society, Prof. Marco Biggiogera underlined that MALDI analyses and imaging promise to be extraordinarily analytical techniques and a new powerful tool for pathologists.

It might therefore be expected that, even in the future, histochemistry will keep a leading position in clinical pathology, and the histochemical journals will continue to be a widely attended forum for both clinicians and basic scientists in the biomedical field.

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