Immunology: Current Research

Immunohistochemistry as an Important Tool for Cancer Immunotherapy: From Diagnosis to Drug Development

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Editorial

Immunohistochemistry (IHC) is a well-established method for identifying both cellular and tissue antigens by means of specific antigen-antibody reactions [1]. This method is a unique analytical tool combining molecular detection with morphological features and can be used on formalin-fixed and paraffin-embedded sections, frozen sections, conventional cytological smears, and other cytological preparations (e.g. isolated circulating tumor cells) [2]. The use of IHC in cancer research has expanded to involve diagnostic classification, anti-tumor drug development, disease prognosis, therapy selection, prediction to response and follow-up, contributing significantly to personalized medicine.

Between the most important applications of IHC in cancer diagnostic are: determination of the cell lineage of undifferentiated malignant tumors, characterization of metastatic malignancies of unknown primary tumors [3,4] and molecular classification of neoplasias [5]. The molecular classification of cancer also permits to identify which patients are most likely to benefit from targeted drugs, leading to a more appropriate therapeutic strategy [6,7]. At present, some IHC-based diagnostic kits are commercially available for the detection of anti-cancer therapeutic targets (e.g. HercepTest™, EGFR pharmDx™, PD-L1 IHC 22C3 pharmDx) and guidelines have been written by expert panels to standardize technique steps, data interpretation and reporting of results [8,9].

In many types of cancer, some IHC analysis such as proliferating antigens, angiogenesis-related molecules, growth factor and their receptors and tumor-infiltrating lymphocytes (immunoscore) are commonly used to obtain information about prognosis as well as a complement to histological grading of tumors [10,11]. Moreover, newer IHC biomarkers, scoring systems and cutoffs are being continuously explored for prediction of outcome [12] or response to molecular-targeted therapies [13,14]. Recently, the immunocytochemical detection of tumor antigens (e.g. PD-1/PD-L1 checkpoint pathway) expressed in circulating tumor cells (CTCs) highlighted the application of IHC into clinical practice permitting the follow-up of patients in a minimally invasive manner [15].

IHC also helps identifying the most relevant animal species with a similar target expression profile to human in order to perform both *in-vivo* toxicity and *in-vivo* pharmacodynamic modeling studies [16]. In combination, these assessments provide useful quantitative information about the effects of cross-reacting biologics predicting potential toxicity in humans. Other application of IHC using animal models is the study of the mechanisms of action for immunotherapeutics [17]. The use of cell lines of known antigens profile expression to induce experimental primary tumors and/or metastasis permit to evaluate, by means of IHC, the anti-tumoral properties of novel biological products as well as the molecular pathways involved in this effect [18].

Some techniques could be used to evaluate the target antigen-binding attributes of biologic drugs (e.g. tissue-based lysate western blots). However, screening studies using IHC provide a unique evaluation combining various immunostaining features such as pattern, distribution, intensity and frequency across panels of frozen tissues from normal humans (tissue cross-reactivity studies). This evaluation permit to identify both previously unknown sites of the target antigen and cross-reactive epitopes (unexpected targets) alerting researches to potential toxicity toward certain organs that could be observed in first-in-human clinical trials [19]. Consequently, tissue cross-reactivity studies constitute an essential part of the preclinical safety assessment package [20,21].

Tissue cross-reactivity studies also permit to compare first generation monoclonal antibodies (usually murine) with the second generation or recombinant versions of these Mabs (e.g. chimeric, humanized) [22]. These studies contribute to detect alterations in the affinity and/or in the specificity of therapeutic drugs when changes in molecule structure, production process or facilities are introduced. In the context of biosimilar antibodies, the IHC comparative studies also allow confirming the pattern of recognition of innovators *vs.* biosimilar candidates [23]. Frequently, IHC using fetal and malignant human tissues permit to obtain a complete characterization about the expression profile of target molecules and binding properties of therapeutic agents [22].

Despite of significant advances in a variety of newer technologies (e.g. next generation sequencing, super-resolution microscopy, proteomic mass spectrometry), up to day IHC remains one of the best choices for both cancer research and routine clinical use [12,13,18]. However, recurrently the confiability and reproducibility of IHC studies are strongly questioned. The lack of standardization of some pre-analytical (e.g. handling of tissue sample, fixation, processing steps), analytical (e.g. antigen retrieval, primary antibody, detection system) and post-analytical (e.g. analysis, data interpretation, final report) factors is not completely resolved. Inconsistencies in IHC studies are considered responsible of both intra and inter-laboratory reported variabilities.

Some recommendations for a better performance of IHC and to recover it reliability include, but are not limited to, adequate use of negative and positive controls in each staining run, application of internal quality control systems for routine immunohistochemical procedures in each laboratory, validation of all immunostaining tests before the introduction into clinical practice, implementation of equivalence studies when deviations during validated technique

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performance occur, harmonization of scores and cutoff points to reduce the inter-laboratory variability in reports and introduction of automated tissue image analysis for an improved signal quantification of the immunohistochemical results.

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