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Review

Garbage in, garbage out: A critical evaluation of strategies used for validation of immunohistochemical biomarkers

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ABSTRACT

The use of immunohistochemistry (IHC) in clinical cohorts is of paramount importance in determining the utility of a biomarker in clinical practice. A major bottleneck in translating a biomarker from bench-to-bedside is the lack of well characterized, specific antibodies suitable for IHC. Despite the widespread use of IHC as a biomarker validation tool, no universally accepted standardization guidelines have been developed to determine the applicability of particular antibodies for IHC prior to its use. In this review, we discuss the technical challenges faced by the use of immunohistochemical biomarkers and rigorously explore classical and emerging antibody validation technologies. Based on our review of these technologies, we provide strict criteria for the pragmatic validation of antibodies for use in immunohistochemical assays.

1. Introduction

The classical method of immunohistochemistry (IHC) allows for visualization of specific antigens in tissues or cells based on antibody-antigen recognition, using brightfield or fluorescence microscopy. The history of IHC goes back to the early 1940s, when Coons and colleagues detected antigens in frozen tissue sections by developing an immunofluorescence technique (Coons et al., 1941). Introduction of a method based on peroxidase-labelled antibodies opened the door to development of more advanced approaches (Mason et al., 1969; Nakane, 1968), enabling IHC to be used on routinely processed tissue sections, such as formalin-fixed paraffin-embedded (FFPE) tissues. However, it took until the early 1990s for the method to become generally accepted in diagnostic pathology (Leong, 1992; Taylor, 1994).

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Table 1 – IHC biomarker assays for FFPE tissues.

Biomarker	Cancer type	Year of approval or clearance	Clinical use
FDA approved single IHC biomarkers			
p63 protein	Prostate	2005	Nuclear basal cell marker for differential diagnosis
c-Kit (CD117)	Gastrointestinal stromal tumours	2004	Diagnosis
Estrogen receptor (ER)	Breast	1999	Prognosis, response to therapy
Progesterone receptor (PR)	Breast	1999	Prognosis, response to therapy
HER-2/neu	Breast	1998	Prognosis, response to therapy

Biomarker assay	Cancer type	Company	Clinical use
Emerging panel-based IHC biomarker assays			
Mammostrat® (p53, HTF9C, CEACAM5, NDRG1 and SLC7A5 IHC combined with a defined mathematical algorithm)	Breast	Clarient InsightDx®	Recurrence risk index for hormone-receptor positive, early stage breast cancer, independent of proliferation and grade
IHC4 (AQUA® Technology combined with ER/PR, HER2 and Ki-67 IHC).	Breast	Genoptix® Medical Laboratory	Recurrence risk assessment

IHC is today a widely used method that can be rapidly performed in most laboratories. The procedure is short, simple and cost-effective. Indeed, IHC has emerged as an important tool to detect cellular markers defining specific phenotypes relative to disease status and biology. Moreover, IHC is utilized for basic and clinical research, from small projects to high-throughput strategies, to evaluate potential biomarkers in clinical patient cohorts. However, the lack of standardized guidelines for determining the specificity and functionality of antibodies renders the translation of promising biomarkers to the clinic difficult. Herein, we discuss the various limitations and technical challenges that need to be addressed when using IHC for biomarker development and clinical validation.

2. Review of clinically used IHC markers approved by FDA

A biomarker is defined as a molecule that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process, or pharmacological responses to therapeutic intervention (Biomarkers-Definitions-Working-Group, 2001). Although great efforts have been made in the last decade to discover novel cancer biomarkers for use in clinical practice, a striking number of these efforts fail to make it into the clinic (Fuzery et al., 2013). One of the causes of this failure of translation could be the limited knowledge that scientists working in biomarker discovery have in analytical, diagnostic and regulatory requirements for clinical assays (Fuzery et al., 2013). Over the last few decades a number of key FDA approved cancer biomarkers have been introduced into the clinic for differential diagnosis of specific tumours, leading to improvement of cancer detection and staging, identification of tumour subclasses, prediction of outcome after treatment, and selection of patients for different treatment options. However, of these approved biomarkers, only five are individual IHC-based biomarkers (Fuzery et al., 2013) (Table 1). The earliest FDA approved biomarkers for IHC application were assays to detect the estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu (c-erbB-2). The presence of these biomarkers in breast cancer tissue serves as a diagnostic, prognostic and predictive method to assist pathologists in identifying breast cancer subtypes and determine whether patients are suitable candidates to receive certain targeted therapies such as Tamoxifen (ER positive patients) or Trastuzumab (Her-2 positive patients). The IHC biomarker c-kit (CD117), which is used in the clinic to detect gastrointestinal stromal tumours (GISTs) (Debiec-Rychter et al., 2004), and p63, which is used to detect the presence of basal cells indicative of normal prostate glands (Shah et al., 2002; Weinstein et al., 2002), are the latest FDA approved single marker IHC-based assays which were approved almost a decade ago in 2004 and 2005, respectively. Since then no other individual biomarker developed for detection in an IHC assays has been FDA approved. However, despite lack of FDA approval, there are many IHC markers utilized in some clinics to assist pathologists in diagnosis and decision making. Such examples include the use of E-Cadherin and/or p120 staining to assist diagnosis of invasive lobular breast carcinoma (Rakha et al., 2010), various antibody panels for diagnosis and sub-classification of malignant lymphomas, as well as the use of the proliferating nuclear marker, Ki67.

An ideal biomarker demonstrating clinical sensitivity and specificity of 100% is almost never achieved in practice due the fact that increasing one of the parameters is only achieved at the expense of the other. As a result, panel biomarker assays are becoming more relevant. Two emerging IHC panel-based assays are Mammostrat by Clarient InsightDx and IHC4 by Genoptix Medical Laboratory. Mammostrat is an IHC-based panel assay that can estimate risk of recurrence in hormone receptor-positive, early stage breast cancer patients which is independent of proliferation and grade. This assay quantifies p53, HTF9C, CEACAM5, NDRG1 and SLC7A5

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