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Original contribution

A validation study of quantum dot multispectral imaging to evaluate hormone receptor status in ductal carcinoma in situ of the breast

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Summary The assessment of hormone receptors, including estrogen receptor and progesterone receptor, has become a standard practice in breast cancer management. However, the need for multiple sections to evaluate each receptor individually by conventional immunohistochemistry may preclude the analysis on some core biopsies with a limited amount of tumors. The aim of the study was to validate the quantitative analysis of nuclear markers estrogen receptor and progesterone receptor by quantum dot–based immunohistochemistry using a multispectral imaging system in ductal carcinoma in situ of the breast. Consecutive sections from a total of 17 cases of ductal carcinoma in situ with excisional biopsies or mastectomies were stained with conventional immunohistochemistry and quantum dot–based, single- and double-labeled immunohistochemistry for estrogen receptor and progesterone receptor. The semiquantitative results from double-labeled, quantum dot–based immunohistochemistry were compared with those from single-labeled, quantum dot–based immunohistochemistry as well as from conventional immunohistochemistry. There was good concordance between double- and single-labeled quantum dot–based immunohistochemistry, and quantum dot–based immunohistochemistry correlated well with conventional immunohistochemistry (Spearman correlation coefficient range from 0.884 to 0.958, $P < .001$). The findings proved the validity and accuracy of quantum dot–based multiplex, multispectral technique in detecting 2 tumor markers in the same cellular compartment simultaneously on a single slide. This technique may enhance our ability to assess multiple breast tumor markers in specimens with limited available tissue. However, several technical and logistic issues await significant improvement before this novel technique can be justified for routine clinical application.

1. Introduction

The presence of hormone receptors, including estrogen receptor (ER) and progesterone receptor (PR), in breast carcinoma is a strong predictive marker for response to hormonal therapy. The assessment of hormone receptors has become a standard practice in breast pathology due to its importance in breast cancer management. Currently, the

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methods recommended by the American Society for Clinical Oncologists and the College of American Pathologists for assessing the receptors are conventional immunohistochemistry (IHC) to detect protein expression and fluorescence in situ hybridization to detect gene amplification [1]. Quantification of ER, PR, and human epidermal growth factor receptor 2 (HER2) immunostaining has been shown to predict response to targeted therapy [2-4]. However, there are problems associated with the current receptor analysis by IHC. In general, IHC stains are performed on serial sections from the same block using single-labeled conventional IHC. One limitation is the need for multiple sections for a complete evaluation of different markers, which precludes analysis on core biopsies with a limited amount of invasive tumors or on microinvasive carcinomas. On the other hand, the numerical standards in the quantitative analysis of receptor IHC (ie, 1% of cells with weak signals for ER/PR-positive results) have also been subjected to interobserver and intraobserver variations by conventional IHC. In fact, IHC with traditional brightfield chromogens is prone to a host of issues that complicate reliable quantitation [5]. Although issues with interobserver variability may be addressed using image analysis systems, most currently available systems require substantial supervision. Such supervision can be labor intensive and, having to repeat it for multiple stained slides, can quickly become cumbersome. A more objective, accurate, and efficient method is highly desired in detecting as well as quantifying hormone receptors and other biomarker expression in breast carcinomas and other tumors.

Commercially available quantum dots (QDs) intended for diagnostic use are cadmium/selenium–based nanocrystal fluorophores coated with a shell of zinc sulfide. They have bright fluorescence, narrow symmetric emission bands and excellent photostability [6]. These characteristics have many significant advantages over conventional fluorescent or chromogenic probes [7]: precise detection of multiple biomarkers simultaneously without the need for computer image overlay or multiple custom filter sets, reinforcement of weak signals, accurate signal quantification, and lack of photobleaching. Using a fluorescent microscope combined with a charged coupled device camera and a liquid crystal tunable filter, QD fluorescent signals can be analyzed by a multispectral imaging (MSI) system such as the Nuance spectral imaging system. In brief, the MSI system can capture an image that includes the entire spectrum for each pixel. Using "unmixing" algorithms, the software can then resolve a mixed overlapping spectrum into its component signals. The less overlap there is between the spectra, the more efficient MSI is in separating the signals for analysis. As natural complements to MSI, QD signals are narrow, strong, and can be designed for minimal overlap. The combination of QD-based IHC and an MSI system has enabled simultaneous labeling of multiple antibodies on a single tissue section with a high fluorescence efficiency as well as subsequent data acquisition and interpretation by an inherent image analysis component [7-10]. Ideally, the multiplex MSI technique may prove to perform as a near-perfect candidate for detection of tumor markers in limited materials, being potentially more accurate, reproducible, and efficient than conventional IHC or immunofluorescence. Recent studies using the QD-based technology have shown promising results for the evaluation of HER2 in breast carcinoma [11,12].

However, given the fact that QDs are essentially a new type of fluorescent probe, serving as detection carriers for the antibodies, the technical issues inherent in multiplex immunostaining in general, such as incompatibility of multiple antigen retrieval and cross-reactivity of multiple antibodies, must also be evaluated to achieve the optimal staining results. Because of the inherent properties of cadmium/selenium/zinc sulfide QDs, there might also be steric or other interference between antibodies and labels, especially when using large QDs. Before embarking on large scale studies and clinical applications, a validation study is required to assess the feasibility, utility, and limitations of multiplexed QD-based IHC in diagnostic evaluation of tumor markers. The aim of this study was to validate the quantitative analysis of nuclear markers ER and PR by QD-based IHC using MSI in breast carcinomas. The study was designed to evaluate the well-circumscribed and easily delineated ductal carcinoma in situ (DCIS) lesions.

2. Materials and methods

2.1. Tissue and case selection

A total of 17 sequential cases of breast DCIS with excisional biopsies or mastectomies were retrieved from the Department of Pathology, Magee-Womens Hospital of University of Pittsburgh Medical Center. Both conventional and QD-based IHC were performed on 4-μm-thick sequential whole sections of formalin-fixed, paraffin-embedded tissue. The study was performed on archival cases of easily identifiable and well-delineated lesions of DCIS to exclude the confounding factor of intermixed benign cells (including ductal cells, lobules, stromal cells, lymphocytes, etc) and to avoid imaging analysis issues with pattern recognition in poorly delineated invasive tumor cells in this pilot quantitative validation study. The study was approved by the Institutional Review Board of the University of Pittsburgh, Pittsburgh, PA.

2.2. Conventional immunohistochemistry

Conventional IHC staining for ER and PR were performed using the rabbit monoclonal antibody clone SP1 (Ventana, Tucson, AZ) and the mouse monoclonal antibody clone PR88 (Biogenex, San Ramon, CA), respectively. At our institution, ER and PR results are reported using a