Scientific Research Publishing

Yangtze Medicine, 2017, 1, 1-7
http://www.scirp.org/journal/ym
ISSN Online: 2475-7349
ISSN Print: 2475-7330

The Application of Quantum Dots Double-Labeling Immunofluorescence Technology in Detection of PR and CD146 in Paraffin-Embedded Tissue Sections of Endometrioid Adenocarcinoma

Jianping Zheng*, Qing Huang, Lei Zhang, Yong Chen, Jinpeng Jiang, Cunjian Yi*

Department of Obstetrics and Gynecology, The First Affiliated Hospital of Yangtze University, Jinzhou, China
Email: *zjpblm@163.com, *Cunjiany@163.com

How to cite this paper: Zheng, J.P., Huang, Q., Zhang, L., Chen, Y., Jiang, J.P. and Yi, C.J. (2017) The Application of Quantum Dots Double-Labeling Immunofluorescence Technology in Detection of PR and CD146 in Paraffin-Embedded Tissue Sections of Endometrioid Adenocarcinoma. *Yangtze Medicine*, **1**, 1-7.
https://doi.org/10.4236/ym.2017.11001

Received: February 23, 2017
Accepted: March 27, 2017
Published: March 30, 2017

Abstract

Objective: The aim was to detect the expression of PR and CD146 in paraffin-embedded tissue sections of endometrioid adenocarcinoma by using QDs double-labeling immunofluorescence, and evaluate the applied value of QDs double-labeling immunofluorescence in endometrioid adenocarcinoma. Methods: To detect the expression of PR and CD146 on 140 cases of paraffin-embedded tissue sections of endometrioid adenocarcinoma by using QDS double-labeling immunofluorescence. Results: The co-expression of PR and CD146 in the endometrioid adenocarcinoma can be detected by QDs double-labeling immunofluorescence, and there was no correlation between them ($P > 0.05$). Conclusion: QDs double-labeling immunofluorescence can detect the localization and co-expression of PR and CD146 in the endometrioid adenocarcinoma.

Keywords

Endometrioid Adenocarcinoma, PR, CD146, QDs Double-Labeling Immunofluorescence

1. Introduction

Endometrioid adenocarcinoma (endometrioid adenocarcinoma) is the most common pathological type of endometrial cancer. Because trend of increasing incidence of endometrioid adenocarcinoma among young patients has been observed in recent years, thus current treatments are focused on the fertility preservation in young patients with endometrioid adenocarcinoma. Although

progesterone therapy may allow some progestrone receptor (PR)-positive patients to retain fertility, there is still no effective method to preserve fertility for the patients with PR-negative tumors. CD146 (cluster of differentiation 146, CD146) is a cell adhesion molecule and belongs to the immunoglobulin superfamily. It has been confirmed that CD146 was highly expressed in a variety of malignant tumors, and was related to poor prognosis of the tumors. Yan *et al* found that CD146 expressed highly in endometrioid adenocarcinoma, and the expression of CD146 was positively correlated with histological grade of endometrioid adenocarcinoma, depth of myometrial invasion, suggesting that CD146 can be used as a potential marker for diagnosis of endometrioid adenocarcinoma [1]. At present, it has been reported that the antibodies against CD146 can inhibit the tumor growth.

Fluorescent semiconductor nanoparticles-Quantum dots (QDs) are composed by elements of type II-VI or III-V with a general size between 1 - 100 nm, and emit fluorescence under stimulation. Because of this unique photophysical property, it begins to be applied in immunofluorescence analysis and achieved promising results. It currently has become a powerful tool for biomedical labeling and optical imaging.

In present study, we detected the expression of PR and CD146 on paraffin-embedded tissue sections by using QDs with different wavelengths (605 nm and 525 nm) and evaluated the effect of QDs based double-labeling immunofluorescence assay.

2. Materials and Methods

2.1. Source of Clinical Specimens

Specimens of 140 female patients (mean age, 48.1 ± 7.5 years) with endometrioid adenocarcinoma were collected from the First Affiliated Hospital of Yangtze University. The specimen collection was approved by the Ethics Committee of Yangtze University and informed consent was obtained from all subjects. These patients did not receive hormone, chemotherapy, radiotherapy and other treatments before sample collection. All specimens were reexamined by an experienced pathologist.

2.2. Reagent

Rabbit anti-human CD146 monoclonal antibody was purchased from Bejing Golden Bridge Biotechnology Co., Ltd. Mouse anti-human PR antibody was purchased from DAKO, QDs double-labeling immunofluorescence kit (QDs-SA-525 nm + QDs-goat anti-rabbit IgG-605 nm) was purchased from Wuhan Jiayuan Quantum Dots Co., Ltd.

2.3. Expression Detection of PR and CD146 Using QDs Double-Labeling Immunofluorescence

Firstly, the paraffin-embedded tissue slice (4 μm) was prepared and followed by deparaffinization, hydration and antigen retrieval. The section was blocked with

2% BSA for 2 h at 37˚C, and incubated with solution containing rabbit anti-human CD146 (1:50 dilution) and mouse anti-human PR antibody (dilution 1: 400) for 2 h at 37˚C in a humidified box. After washing the sample three times with PBS-T (5 min for each wash), the biotinylated goat anti-mouse IgG (1:300 dilution) was added and incubated for 45 min at 37˚C in a humidified box, then washed three times with PBST. After that, the solution containing quantum dot-SA complex (1:30 dilution) and quantum dot-goat anti-rabbit IgG complex was dropped and incubated 2 h at 37˚C., and then the sample was washed three times with PBS-T (5 min/wash), followed by twice TBS wash (5 min/wash). Finally the section was sealed with buffered glycerol sealing agent and observed under a fluorescence microscope (Olympus). QDs-SA-525-labeled antigen was shown with green fluorescence signal, while QDs-goat anti-rabbit IgG-605-labeled antigen was shown with red fluorescence signal.

3. Result

The co-expression of QDs immunofluorescence double labeled-PR and CD146 in 140 cases of endometrioid adenocarcinoma was observed by fluorescence microscope, and shown under ultraviolet excitation: the expression of PR in endometrioid adenocarcinoma was shown with green fluorescence under the blue background. The positive signals were localized in the normal glandular epithelium and tumor cell nucleus. The expression of CD146 in endometrioid adenocarcinoma was detected and shown as the red fluorescence, and the positive signals were localized in plasma membrane and/or cytoplasm of tumor cells, interstitial vascular endothelial cells and fibroblasts. Among them, 40 cases showed positive expression for both PR and CD146 (Figure 1(A)), and 25 cases of samples

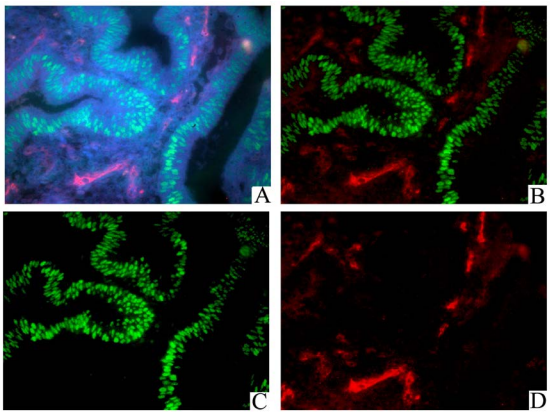

Figure 1. Co-expression of PR and CD46 in the endometrioid adenocarcinoma by QDs double-labeling immunofluorescence (×400, UV excitation). (A) The original image of co-expression of PR and CD146 in endometrioid adenocarcinoma (×400, UV light excitation); (B) Co-expression of PR and CD146 after removal of tissue background (×400, ultraviolet light); (C) Separated image for PR expression (×400, UV light); (D) Separated image for CD146 expression (×400, UV light).

showed negative for both PR and CD146 expression, and there was no correlation between them ($P > 0.05$) (Table 1).

When the localization of PR and CD146 is same or close, the Nuance FX multispectral imaging system can be used to unmix each signal, which can remove the autofluorescence of the tissue and improve the signal-to-noise ratio of the image. Figure 1(B) showed a co-expression image of PR and CD146 without tissue background, C showed single image of PR expression, and D was an image of the single CD146 expression. Compared with A, the signal- to-noise ratio of the images was significantly improved after removing the autofluorescence of tissue background. Using the Nuance FX multispectral imaging system, we can also transform the intensity of the subjective fluorescence signal into an accurate spectral signal, and also analyze the strength of their expression and co-expression by quantitatively calculating the content of the regions. We further quantified the whole spectrum. In the labeled region, the PR region signal was 37,960, the total signal was 3,297,159; the CD146 region signal was 26,866 and the total signal was 2,069,452 (Table 2).

4. Discussion

The correct diagnosis and classification of tumors is the basis for effective individualized treatment of patients, while current diagnosis of tumor is mainly dependent on medical imaging, biochemical analysis, cytopathology test and immunological tests for tumor markers. Immunohistochemical technique has been widely used in pathological diagnosis because of its specificity, high sensitivity and accurate positioning, and the combination of morphology and function. However, it also has some disadvantages such as many interference factors, large error of detection and low objectivity of result judgment. More importantly, the immunohistochemical technique is considered semi-quantitative and difficult to accurately reflect the differences among individuals [2] [3], thus it cannot be

Table 1. The expression correlation of CD146 and PR in endometrioid adenocarcinoma (n, %).

	Case number	CD146		X2	P	Correlation coefficient
		Positive	Negative			
PR				0.984	0.321	0.084
Positive	78	40 (51.28)	38 (48.72)			
Negative	62	37 (59.68)	25 (40.32)			

Table 2. The quantitative result of QDs immunofluorescence double labeled-co-expression of PR and CD46 from Figure 1.

	Marker Area (pixels)	Total Signal (counts)	Avg Signal (counts)	Standard Deviation (counts)
PR	37,960	3,297,159	9.11	20.24
CD146	26,866	2,069,452	5.72	14.00

used to guide individualized treatment. In addition, with the development of medicine, the proteome in the disease process can be better understood, and a variety of imaging platforms for disease-related proteins are also increasingly important, whereas the application of multiple immunohistochemistry in formalin-fixed, paraffin-embedded tissue is limited. Furthermore, immunofluorescence techniques can also be used to explore the distribution of various proteins at cellular and subcellular levels. However, the traditional organic fluorescent groups have deficiencies such as luminous short time and easily quenched. In addition, detection of multiple proteins on a paraffin-embedded tissue using a laser confocal microscope usually requires a multiple laser beams of different wavelengths to excite, and most of the bandpass filters are too wide to separate these fluorescent emitting groups with close spectra.

Compared with the traditional fluorescent molecules, QDs have the following advantages: 1) broad and continuous excitation spectrum, and narrow and symmetrical emission spectrum; 2) strong Stokes shift; 3) strong Light stability. As the quantum dots have a strong light stability, it has a very long fluorescent life and cannot easily be photolysed or bleached, and can be excited multiple times. Thus it is convenient for observation of labeled markers with a long time, as well as short term storage, which is beneficial to clinical research. In addition, because of its narrow and well-balanced emission spectrum, it is possible to use a single excitation light source to simultaneously excite quantum dots of all colors. Thus it can be used for multiple labeling of subcellular molecules. It is particularly important for analysis of a large number of parameters for complex specimens. PAN Qi *et al.* simultaneously detected the co-expression of EMMPRIN and P53 proteins in human lung cancer tissue microarrays by using QDs double-labeling immunofluorescence, and they also detected their localization in the same cell under the same excitation wavelength, but there were some overlapping signals in certain regions. They further used multi-spectral imaging separation technology (United States Cambridge Nuance TM) to separate the pre-collected signals and then reconstructed the image, this technology provided good result and the image can also be used for quantitative analysis [4]. We detected the co-expression of PR and CD146 in paraffin-embedded endometrioid adenocarcinoma tissue using QDs double-labeling immunofluorescence technology, the positive expression of the QDs-SA-525 labeled PR was indicated by green fluorescent signal, while expression of QDs-goat anti-rabbit IgG-605 labeled CD146 was shown by red fluorescent signal. It was shown that different expression of PR and CD146 could be clearly observed under the same wavelength of excitation light, but there was no significant difference and correlation between the expression of PR and CD146. This technology offered real-time imaging results for the fluorescence labeling experiments without the needs of image overlapping and other processing. In addition, it simplified the image capture for multi-tagged results and reduced biases caused by the image processing. Compared to the conventional immunohistochemical staining or immunofluorescence imaging techniques, the availability of spectral analysis is

another great advantage of QDs imaging. Spectral analysis further enhances the advantages and confidence of QDs in multicolor imaging, which makes it possible to quantify molecular imaging.

Although QDs have unique advantages in the field of biomedical applications, there are still many issues: 1) the toxicity of QDs. Although QDs can be used for in vivo imaging, this technology need to be performed in vivo, thus the safety is a focus of this technology. The heavy metals that make up the core of QDs are toxic and the formed compounds (Cd Se) may also have cell and target organ toxicity. Although its outer shell structure can reduce its toxicity, some coatings of QDs are also toxic. In addition, because different components (coatings and dissolved chemicals, etc.) may show the different toxicity, thus the potential toxicity of QDs becomes difficult to assess due to the diversity of QDs. For example, Kim J *et al*. found that the different toxic effects of QDs resulted in different hemorheological properties for red blood cells, including hemolysis, deformation, aggregation, and morphological changes when cells were exposed to three different forms of graphite QDs (non-functional, hydroxylated and carboxylated). In addition, they found that the changes of hemorheological indicators can be used to evaluate the cytotoxicity of QDs [5]; 2) QDs blinking. Cichy B *et al*. found a large deviation between their experimental results and the usual results due to the appearance of QDs blinking. By using single-particle spectrum, they studied the two different blinking mechanisms related to the chare inbalance in the AgInS2 and AgInS2/ZnS responsible for fast non-radiative relaxation and surface recharging of the QDs [6]; 3) Large size of QDs. QDs are larger than conventional fluorescent dyes, and will be larger after being modified and result in a more significant spatial steric hindrance, which will to a certain extent limit its application in molecular biology. Howarth M *et al*. have found that large QDs were difficult to enter the synapses in hippocampal neurons [7]; 4) The yield of QDs is influenced by the physical and chemical states of QDs. The different surface modification and cross-linking can affect the physical and chemical states of QDs, thus reducing the quantum yield [8].

In sum, although QDs have its unique advantages, there are also some shortcomings. Thus it is important to solve the existing problems when we utilize its advantages to work for us at the same time, and further making the QDs better for us to use.

Acknowledgements

This work was supported by a grant from the Outstanding Medical Academic Leader Program of Hubei Province (Hubei Health and Family Planning Bureau (2013) NO.4).

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