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Mueller polarimetric imaging for characterizing the collagen microstructures of breast cancer tissues in different genotypes

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ABSTRACT

Mueller polarimetric imaging has been proven a valid method for detecting tissue in biomedical field. In the present study, we applied the Mueller matrix microscopy to characterize the microstructures of breast cancer tissues in different genotypes. The experimental results demonstrated that Mueller matrix parameters were effective in distinguishing the genotypes of breast cancer, especially for triple-negative type. Then, we conducted quantitative analysis of Mueller matrix and immunohistochemistry (IHC) experiments. On that basis, we verified the relationship between collagen structures and polarization properties as well as the remodeling of collagen structures in breast cancer, which could provide new evidence for using high invasiveness and metastatic dissemination as triple-negative breast cancer. In comparison with traditional methods, Mueller polarimetry is independent of complex tumor marks. Besides, it is quick, sensitive and non-stained. Thus, it has a potential in the application for clinical diagnosis and is expected as a new method for researching the pathological process of breast cancer.

1. Introduction

Breast cancer has been one of the greatest threats to the health, taking up 15% of cancer deaths in women around the world [1–3]. Reasonable classification of breast cancer which is a highly heterogeneous tumor is of huge significance to treatment and prognosis. Under different genetic and epigenetic changes, there are above 20 distinct histopathological subtypes of breast cancer [4]. The genotype of breast cancer is as important as morphological classification, which is indispensable in clinical practice [5]. Based on the expression of estrogen receptor (ER), progesterone receptor (PgR) and overexpression of human epidermal growth factor receptor 2 (HER2), the breast cancer can be classified into four genotypes: two of these are classified as ER-positive tumors (Luminal-A and Luminal-B), and the other two classified as ER-negative tumors (triple-negative and Her-2 positive) [6]. Comparatively, triple-negative breast cancer has higher proportion in African–American women and it has the lowest five-year survival rate from many cancer statistics. Furthermore, the tumor marks of triple-negative breast cancer are uncertain, which makes accurate clinical diagnosis more difficult to achieve [7].

Immunohistochemistry (IHC) is now the most common method to detect the genotyping of breast cancer [8]. Yet the preparation of an IHC sample is complicated and time-consuming. In the meantime, the high cost is a major drawback in clinical of IHC. Recently, various optical techniques for tumor detection have been developed. For instance, Ahmad Golaraei et al. reported that polarimetric second harmonic generation (SHG) microscopy could be used to reveal the variations of collagen ultrastructure in the extracellular matrix (ECM) in various genotypes of breast cancer [9]. Alessandra Tata et al. proved that wide-field tissue polarimetry could identify the tissue of breast cancer margin effectively [10]. Dong et al. demonstrated that Mueller matrix microscope could help distinguish human breast ductal cancer at different stages [11].

Mueller matrix imaging was proven as a promising optical technique for the detection of microstructure in different types of cancer tissue, especially for anisotropic sample [12,13]. Besides the high sensitivity, Mueller matrix imaging has many other advantages. For instance, the detect samples (tissues, cells and other biological specimens) are label-free, and the detection process is easy to operate [14,15]. Several types of cancer originating from the superficial epithelium, including liver cancer [16,17], skin cancer [18], cervical cancer [19], etc. [20,21], can be effectively diagnosed under Mueller matrix polarimetry.

It is reported that the extracellular matrix (ECM) of breast cancer cell and breast cancerous tissue undergoes variations [22], the variations of collagen ultrastructure in ECM. As the major component of ECM, the collagen components of cancerous are significantly different from normal tissue [9,23,24]. Type I collagen is the most common

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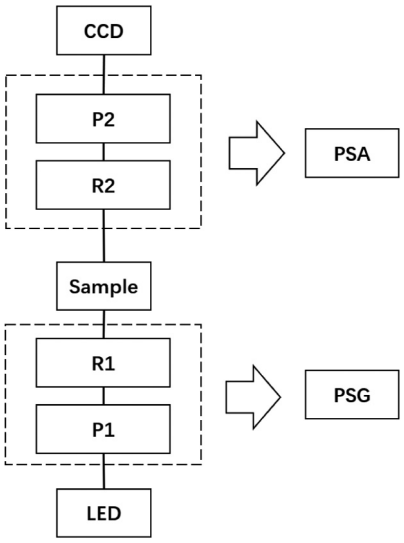

Fig. 1. Schematic of the Mueller matrix microscope: R1, R2: quarter wave-plate; P1, P2: polarizer; PSG: polarization state generator; PSA: polarization state analyzer.

and abundant ECM component in breast cancer tissue. The dynamic modeling of the triple helix structure of type I collagen contributes to the formation of tumor [25]. Furthermore, experimental evidence has proven that there is derangement of type I collagen fibers in breast carcinoma, as well as the overexpression in malignant tumors [26,27]. Due to the variations in density and component of collagen fibers in breast cancerous tissue [28,29], as well as the high sensitivity of fiber changing [30], Mueller matrix microscopy will act as an effective tool to distinguish different types of breast cancer.

In the present study, we selected four genotypes of breast cancer tissue samples and measured their microscopic Mueller matrix images. Next, we calculated the polarization parameters using Mueller matrix polar decomposition (MMPD) and Mueller matrix transformation (MMT). Subsequently, we analyzed the Mueller matrix quantitatively by transforming it into frequency distribution histogram (FDH) and central moment parameters. Finally, we verified the previous results by performing biological experiment. The results suggested that the Mueller matrix and parameters derived from it could be a potential non-tumor markers and non-stained method for differentiating the genotypes of breast cancer, especially for triple-negative type. In the meantime, it was proven that polarization was closely associated with the remodeling of collagen, which is of great significance to researching the variations in collagen structure of breast cancer.

2. Methods and materials

2.1. Experimental setup

As shown in Fig. 1, the Mueller matrix microscope we used is based on a commercial transmitted-light microscope (L2050, Liss Optical Instrument Factory, Guangzhou, China) by adding the polarization state generator (PSG) and analyzer (PSA). The light beams from the LED (3 W, 632 nm, $\Delta\lambda = 20$ nm) pass through the PSG which consists of a polarizer (P1, extinction ratio 500:1, Daheng Optics, China) and a rotatable quarter-wave plate (R1, Daheng Optics, China). And then the light beams transmit the tissue sample, the objective lens (10×, 4×) and the PSA which consists of another quarter-wave plate (R2, Daheng Optics, China) and polarizer (P2, extinction ratio 500:1, Daheng Optics, China). Finally, the light beams reach to the CCD camera (QImaging 74-0107A, Canada) and be recorded.

2.2. Mueller matrix polar decomposition and Mueller matrix transformation parameters

Previous studies have demonstrated that Mueller matrix can reveal the total polarimetric information of samples. However, the individual element of Mueller matrix lack explicit physical meaning, so we need to use several methods to extract the structural information of matrix and obtain polarimetric parameters connected with the sample. Lu and Chipman proposed the Mueller matrix polar decomposition (MMPD) in 1996 [31], which is based on three matrices representing independent polarimetric properties of the sample. In this study we use the linear retardance (δ) derived from the decomposed Mueller matrix shown as Eq. (1).

$$M = M_\Delta M_R M_D \tag{1}$$

In Eq. (1) M_Δ is the depolarization matrix, M_R is the retardation matrix and M_D is the diattenuation matrix.

Through the studies on fibrous scattering samples, He et al. proposed the Mueller matrix transformation (MMT) process to extract several parameters related to the structural features of the samples [32]. The parameters of MMT used in this study are defined in Eq. (2) and the MMPD parameters are defined in Eq. (3).

$$t_2 = \frac{\sqrt{(m42)^2 + (m43)^2}}{2} \tag{2}$$

$$\theta = \frac{1}{2}\tan^{-1}\left[\frac{r_2}{r_1}\right] \tag{3}$$

In Eq. (3), *r1* and *r2* are the elements of the vector of retardance.

In summary, the MMPD parameter *t2* is closely related to the birefringence of the tissues, while the MMT parameter θ is related to the orientations of the fibrous structure.

2.3. Central moment analysis

To analyze the Mueller matrix elements quantitatively, we adopt the central moment method derived from FDHs [33]. For a random variable X, its central moments expected value, variance, skewness, and kurtosis are defined by Eq. (4).

$$\begin{aligned} &P1 = mean = E(X) \\ &\mathrm{P2} = \text{standard deviation} = \sqrt{V\mathrm{ar(X)}} \\ &\mathrm{P3} = \text{skewness} = \frac{E(X-\mu)^3}{\sigma^3} \\ &\mathrm{P4} = \text{kurtosis} = \frac{E(X-\mu)^4}{\sigma^4} \end{aligned} \tag{4}$$

In Eq. (4), P1(μ) is the mean value of FDH and P2(σ^2) is the variance. P3 (skewness) represents the asymmetry of the FDH, while P4 (kurtosis) indicates the "peakedness" of the FDH. Among them, P1 is closely related to the type of anisotropy. P2 is sensitive to the complexity of the microstructure in the samples. P3 is related to the heterogeneity of the samples and P4 represents the concentration of data. Moreover, P2, P3, and P4 are insensitive to sample orientation directions [34]. In this work, we first transfer the Mueller matrix images to FDHs, and then calculate the central moment of FDHs to analyze the Mueller matrix quantitatively.

2.4. Cell culture and tumor inoculation

The cell lines used in this experiment were as follows: MCF-7(Luminal-A), BT-474(Luminal-B), SK-BR-3(Her-2) and MDA-MB-231(Triple-Negative), which were purchased from Shanghai cell bank of the Chinese Academy of Sciences and cultured in the recommended media.

Four types of cell lines were digested by trypsin with EDTA and cell numbers were determined by cell counter. The cells were centrifuged and resuspended in a concentration of 6×10^7/ml. Each group