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Study of the molecular variation in pre-eclampsia placenta based on micro-Raman spectroscopy

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Abstract

Purpose   Study of the molecular variation in pre-eclampsia placenta based on micro-Raman spectroscopy.

Methods   Five pregnant women with pre-eclampsia from Nanfang hospital were selected as study group whose average age is 28.5 years and 38 ± 2 weeks gestation. The same period of healthy pregnant women, whose average age is 27.6 years and pregnant 39 ± 1 weeks, as control group ($n = 5$). The normal and pre-eclamptic placental tissues are detected by micro-Raman spectroscopy with the spectrum resolution of 1 cm^{-1}.

Results   We find that the protein structure of α-helix, β-pleated sheet and β-turn is overlying in pre-eclamptic placenta, which lead to a disorder of protein structure. The Raman peaks assigned to tryptophan indole ring and phenylalanine in pre-eclamptic placental tissue are more higher than that in normal tissue.

Conclusions   Results suggest that the ordered structures of the main chain in protein molecules are reduced significantly, and the amino acid of side chains is damaged obviously. And a principal component analysis is used to classify the Raman spectra between normal and pre-eclamptic placental tissues. This study presents that Raman spectroscopy has a great potential on the mechanism research and diagnosis of placental lesions.

S.-J. Chen and Y. Zhang contributed equally to this study.

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Introduction

Pre-eclampsia (also known as pregnancy-induced hypertension, PIH) is a disorder that generally develops late in pregnancy and is characterized by a sudden onset of high blood pressure, edema and protein in the urine [1]. However, the immunity, impaired vascular endothelial cells and insulin resistance etiology in this disease are unclear [2]. Researchers are interested in the etiology and pathology of pre-eclampsia. Turner et al. [3] observed that the concentration of tyrosine, histidine, and phenylalanine in pre-eclampsia placenta was higher than normal tissue. And Raouf et al. [4] reported that there was a decreased intensity at the protein bands from the Fourier spectra of lyophilized serum samples. There were some conflicting views in the previous studies [3, 4]. Therefore, an in-depth research on the molecular and protein variation of pre-eclampsia is urgently needed for scientists.

Confocal micro-Raman spectroscopy, which depends on polarizability change of an oscillating molecule, has proven extremely versatile and has led to a vast array of

applications across the disciplines of chemistry, physics, biology, biomedicine, engineering, and archeology. With an insensitivity to the presence of water, Raman spectroscopy is a particularly attractive technique for life sciences. In addition to these, compared with other optics technologies including second harmonic generation, Fourier transform infrared spectroscopy (FTIR), and multiphoton microscopy, Raman spectroscopy requires simple sample preparation and contains abundant information [5–8]. With the help of these superiorities, Raman spectroscopy has been employed to detect the damage of gestational diabetes on placenta [9]. However, there were few reports about placenta in pre-eclampsia. In this study, the micro-Raman spectra were employed to characterize the molecular variation in pre-eclamptic placenta tissues.

Materials and methods

Sample collection and preparation

Informed consent for the study was obtained from all the women concerned, and approval was given by the Ethics Committee of the Faculty of Medicine, Nanfang Hospital, Southern Medical University. In this study, five pregnant women with pre-eclampsia from Nanfang hospital were selected as study group whose average age is 28.5 years and 38 ± 2 weeks gestation. The same period of healthy pregnant women, whose average age 27.6 years and pregnant 39 ± 1 weeks, as control group ($n = 5$). All of the placentas were obtained immediately after cesarean deliveries. There were no other obstetric complications in the two groups of pregnant women.

Experiment

Tissue samples were placed on a silicon slice for measurement of Raman spectroscopy because there were no extra Raman peaks for the silicon slice in the fingerprint spectrum region from 700 to $1,800 \text{ cm}^{-1}$. The Raman spectra were acquired using a Renishaw (New Mills, UK) inVia confocal micro-Raman spectroscopy system. The samples were excited by 785 nm laser and after attenuation through prisms and filter, the power of the laser exposed on the samples was 9 mW. Spectra were obtained from tissues with a $20\times$ optimized objective and the signal was integrated for five times over a spectral range of 700–$1,800 \text{ cm}^{-1}$. Peak frequencies are calibrated with the silicon at 520 cm^{-1}. For each sample, at least 20 Raman spectra are obtained. All the data are collected under the same conditions. Tissue samples were placed on a silicon slice for Raman measurement. In order to compare the related spectra changes, the phenylalanine band ($1,004 \text{ cm}^{-1}$) was chosen to normalize

the spectra. The final Raman spectra are baseline corrected by the software R 2.8.1, and smoothed, normalized, and averaged by ORIGIN PRO 8.5 (OriginLab Corporation, Northampton, MA, USA), together with the Raman spectroscopic software WIRE 3.2.

Data analysis

The spectra recorded from normal and pre-eclamptic placental tissue were analyzed statistically using principal component analysis (PCA) by Matlab [6, 10, 11]. The analysis is oriented toward modeling a variance–covariance structure of a data matrix from which the eigenvalues, corresponding to principal components, are extracted. Each principal component (PC) is a linear combination of the n independent wavenumber variables $x_1, \ldots, x_n$. So, for example:

$$PC1 = a_1 x_1 + a_2 x_2 + \cdots + a_n x_n.$$

The first PC accounts for the greatest variance, and so corresponds to the largest eigenvalue. The second PC is orthogonal to the first, with each successive PC being both orthogonal to all those preceding, and accounting for a decreasing proportion of the variance. In this paper, we choose the first three PC for analysis (Fig. 1).

Results

A typical Raman spectrum of normal placenta is shown in Fig. 2. We can find that for normal placenta, primary Raman peaks were observed at 758, 940, 1,005, 1,033, 1,343, 1,453, 1,605, 1,620 and $1,663 \text{ cm}^{-1}$. The Raman peak of $1,453 \text{ cm}^{-1}$ belonged to CH_3 (CH_2) deformation vibration of proteins. While $1,663 \text{ cm}^{-1}$ is assigned to amide I, the bands at 758 and $1,343 \text{ cm}^{-1}$ should be assigned to tryptophan
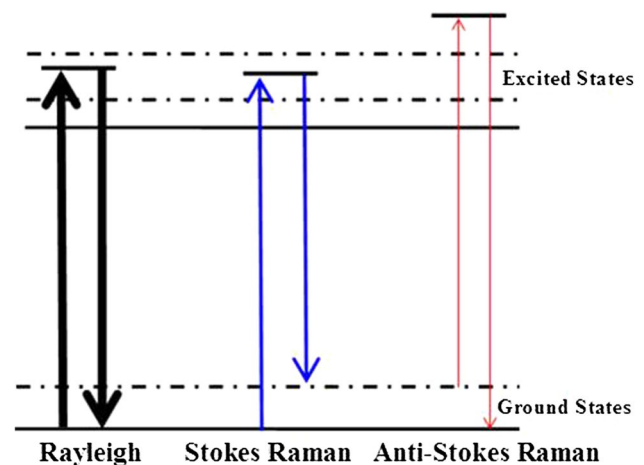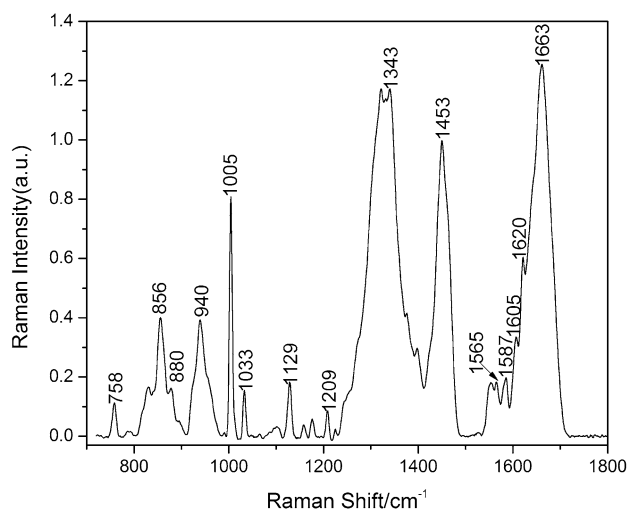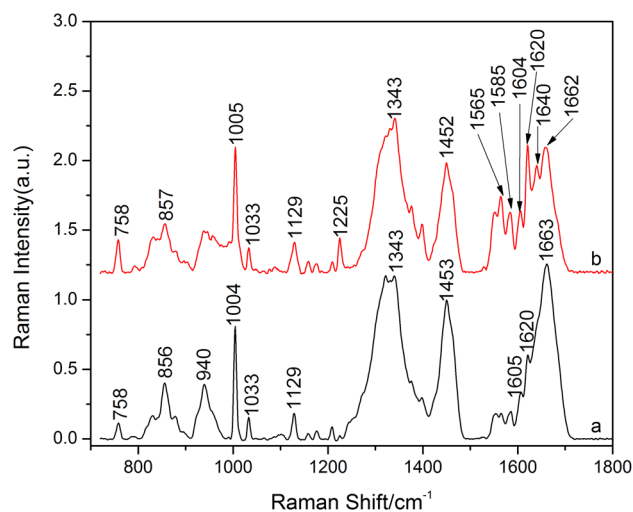

Fig. 1 Diagrammatic Raman scattering effect

Fig. 2 The Raman spectrum of normal placenta

Fig. 3 The Raman spectra of normal and pre-eclamptic placenta. **a** Normal placenta. **b** Pre-eclamptic placenta

Table 1 Raman shift and the tentative assignment of normal placenta

Frequency (cm^{-1})	Assignment	Frequency (cm^{-1})	Assignment
758	Trp	856	Tyr
880	Trp	940	ν_s (PO$_3^{2-}$)
1,005	Phenylalanine	1,033	Phe
1,129	ν (C–N)	1,159	δ (C–O)
1,175	Tyr, Phe	1,209	Tyr
1,343	Trp	1,399	His
1,453	δ (CH$_2$)	1,549, 1,565	Trp
1,585	Trp	1,605	Phe
1,620	Phe	1,663	Amide I protein

ν stretch, ν_s symmetry stretch, *Trp* tryptophan, *Tyr* tyrosine, *Phe* Phenylalanine, δ deformation, *His* histidine

indole ring, and Raman peaks at 1,005, 1,033, 1,605 and 1,620 cm^{-1} belong to phenylalanine. And the assignments of all the main Raman peaks are shown in Table 1.

This study mainly discusses the differences in the region of 1,500–1,700 cm^{-1} in Fig. 3. Figure 3a depicts Raman spectrum of normal placenta while Fig. 3b shows the Raman spectrum of pre-eclamptic placenta. There are many differences between the Raman spectra of pre-eclamptic and normal placenta tissue. By contrast in Fig. 3, we can see that the intensity of peaks at 1,005, 1,605 and 1,620 cm^{-1} increased which belong to phenylalanine, while 785 and 1,585 cm^{-1} also enhanced which assigned to indole ring of tryptophan. The spectral line of amide I at 1,663 cm^{-1} from normal placenta presented α-helix structure, but in the Raman spectrum of pre-eclamptic tissue, peaks at 1,662 and 1,640 cm^{-1} showed a overlying of α-helix, β-pleated sheet and β-turn.

Finally, PCA was used to distinguish the Raman spectra of normal and pre-eclamptic placental tissues, which is shown in Fig. 4. In this section, 21 Raman spectra of pre-eclamptic placental tissue and 20 Raman spectra of normal placenta were used for PCA. We formed the variance–covariance matrix of the dataset, in which the eigenvalues or factors corresponding to PCs were extracted and the resulting scores were noted. The panel in Fig. 4 shows a plot of sample number vs. scores of PC1 vs. PC2, which represents the discrimination between spectra of normal and pre-eclamptic placental tissues. This plot clearly shows that most of the normal placentas lie in the first and fourth quadrants while most of the pre-eclamptic placental tissues lie in the second and third quadrants except two points. It could give us a useful help on qualitative distinction the normal and pre-eclamptic placental tissues.

Discussion

By comparing the Raman spectra of normal and pre-eclamptic placental tissues, it was clear that the characteristic spectral line of amide I at 1,663 cm^{-1} from normal placenta assigned to α-helix structure, but peaks at 1,662 and 1,640 cm^{-1} in pre-eclamptic placental tissue showed a superposition composed by α-helix, β-pleated sheet and β-turn which led to a disorder of protein structure [12]. Meanwhile, the Raman spectra also showed variations of amino acid residue on the protein side chain. In pre-eclamptic placenta, we observed significant enhanced spectral lines of tryptophan indole ring and phenylalanine and some special lines at 1,399 and 1,640 cm^{-1} which cannot be found in normal placental tissue, which shows an addition
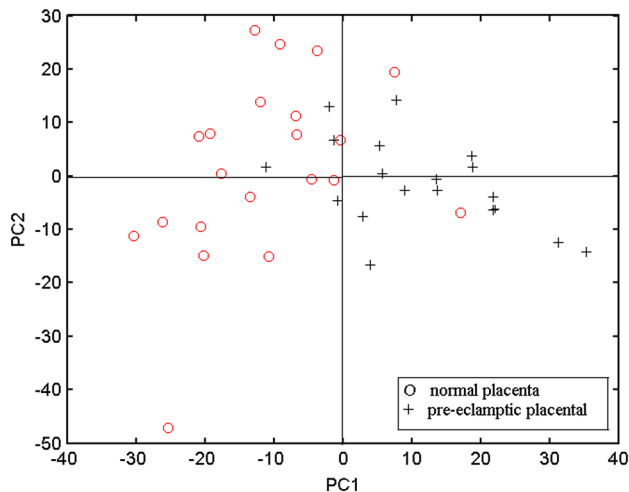
Fig. 4 Score plots of PCA

of the content of amino acid residue in pre-eclamptic placenta. The vasoactive substances synthesized and secreted by hypoxic–ischemic placenta (such as soluble Fms-like tyrosine kinase 1, cytokines, angiotensin II type 1 receptor activating antibody and thromboxane) entered into the maternal blood circulation and caused vascular endothelial cell dysfunction, which can further promote the release of vasoactive substances to decrease the activity of NO and increase reactive oxygen species and free radicals. Oxidation changed the main chain conformation and injured the side chain groups of protein which presented characteristic enhanced spectral lines of phenylalanine at 1,005, 1,605 and 1,620 cm^{-1} and those amines with activity side chain such as tryptophan indole ring at 758 and 1,585 cm^{-1} [13, 14].

Conclusion

In this study, the results show that: (1) the protein structure of α-helix, β-pleated sheet and β-turn is overlying in pre-eclamptic placenta, which lead to a disorder of protein structure. (2) The Raman peaks assigned to tryptophan indole ring and phenylalanine in pre-eclamptic placental tissue are more higher than that in normal tissue. It suggests that the ordered structures of the main chain in protein molecules are reduced significantly, and the amino acid of side chains is damaged obviously. (3) The PCA could give us a useful help on distinguishing the Raman spectra between normal and pre-eclamptic placental tissues. And the Raman spectroscopy presents a great potential on the mechanism research and diagnosis of placental lesions.

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