Raman spectroscopic study on Hela cells irradiated by X rays of different doses

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Raman spectra are used for studying the structure and protein, nucleic acid, lipid, and carbohydrate contents, while cervical cancer cells irradiated by X rays of different doses are cultivated for 24 h. After irradiation by X rays, the following results are obtained. (1) Some 12-Gy groups move to the 1237-cm^{-1} band in compared with the control group's 1240-cm^{-1} band; after irradiation by 6-Gy X ray, the 1662-cm^{-1} band of amide I has a blue shift of 10 cm^{-1}. The above two parts show that because of X ray irradiation, some proteins' random coil structures have transformed into β folding. (2) The 759-cm^{-1} band disappear in the 6-Gy group; the 570-cm^{-1} band of every group has a red shift, but the changes in intensity are different; the 1335-cm^{-1} band in every group has a blue shift, and all their intensities increase. These show that although the 570-, 759-, and 1335-cm^{-1} bands all belong to the tryptophan residue indole ring vibration, the molecular vibration energy structures which produce scattering lights are different. (3) The 786-cm^{-1} band only has a blue shift of 3 cm^{-1} in the 6-Gy group, and the non-hydrogen band of the phosphoric acid diester (O−P−O) increases. The frequency deviation of the 1089-cm^{-1} band is erratic, and the bent symmetry stretch vibration conformation of phosphoric acid diester key (O=P=O) in the nucleic acid is complex. (4) The 1570-cm^{-1} band has a blue shift, and its intensities all decrease, while the C=C conjugated duplet bond oxidizes, and the content of C=C decreases.

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Cervical cancer is the second most common cancer in women worldwide, and is the leading cause of cancer mortality in women. It has been reported that about 500000 new cervical cancer cases appear every year worldwide. Cervical malignancy is preceded by cervical intraepithelial neoplasia (CIN) I, CIN II, CIN III and carcinoma *in situ* before becoming invasive[1]. Radiotherapy, surgery, and chemotherapy, alone or in combination, are the accepted modalities of treatment for cervical malignancy.

X ray is a short electromagnetic wave. When carcinomatous cells are irradiated by X rays, some proteins, nucleic acids, and intracellular lipids are damaged or repaired. Synchronously, X ray radiation also induces the startup of some apoptotic mechanisms[2−5]. Consequently, X ray has been continuously used to cure malignant tumors in the clinical setting. In the clinic, however, there remains no scientific dose reference for X ray irradiation in cervical cancer therapy. Moreover, the design of the irradiation dose is always according to the experiences of individual doctors, which is quite subjective.

Due to its sensitivity, spectroscopic analysis is an effective method of analyzing structures, components, and contents of various materials[6−8]. Cervical cancer has been one of the well-studied forms of malignancy using optical spectroscopic methods, mostly by Fourier transform infrared spectroscopy (FTIR), fluorescence, and a combination of reflectance and fluorescence spectroscopy. Raman spectroscopy offers certain distinct advantages over these techniques, including high spatial resolution (down to 1 mm), because it requires less sample preparation, it is not influenced by water bands, and can be used for *in vivo* or *in situ* measurements. Thus, we employ the Raman spectroscopic technique to study the changes in structures and contents of Hela cells, and hypothesize that the research could provide a reference dose (RD) of X ray irradiation for human cervical carcinoma therapy in the future.

The Hela cell line for this study was obtained from the cell storeroom of Shanghai. The cell line was maintained in an RPMI1640 medium (Gibco Co., USA), supplemented by 10% heat-inactivated fetal bovine serum (Gibco Co.) at 37 °C in a humidified 5% CO_2 atmosphere. The cells were harvested in exponential growth phase from the culture medium, and counted and replanted in 7 culture dishes (the number of cells in each culture dish is the same). These 7 groups were divided into 6 different X ray irradiation dose groups (1, 3, 6, 8, 10, and 12 Gy) and a control group (0 Gy) for X ray irradiation. Every group included three parallel groups.

The X ray irradiation was carried out at room temperature using an X-ray generator (6-MeV linear accelerator, Siemens Co., Germany), where the source skin distance (SSD) was 100 cm, and the irradiation area was 10× 10 cm^2. These cells were then cultivated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h sequentially. The 7 culture dishes were then taken out for harvest, counting, gentle washing in phosphate buffer saline (PBS) 3 times (each group had the same cell number of 10^6), and correspondingly fixing them with alcohol (95%) on the slides. Lastly, Raman spectra in the 2000−500 cm^{-1} range were collected by laser confocal microscopy Raman spectra apparatus (made in Renishaw company, UK) where three different observation points were altered to prevent the nonuniform scattering of facula. The excitation wavelength of this apparatus was 532 nm, and the scanning

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time was 20 s. The Raman spectra are shown in Fig. 1 while the Raman spectra assignations of the Hela cells are shown in Table 1. As shown in Figs. 2, 3, and Table 2, we drew figures of relative intensity, which showed some peak intensities (1662, 786, 1089 cm^{-1}) divided by the 1005-cm^{-1} peak in every group. Table 3 shows the scattering bands in the Raman spectra.

The protein main-chain's conformation primarily came from the peptide bond, the C−C skeleton, and the C−N skeleton. The frequency shifts and intensity changes of the amide I and III bands related to the secondary structure of the peptide bond. This could be sensitively used for the protein's secondary structure study. In the Raman spectrum of the control group, the 1240-cm^{-1} band came from the amide III random coil, and the 1662-cm^{-1} band came from the amide I random coil[9] (Table 1). The peak of 885 cm^{-1} was derived from the stretch vibration of the C−C skeleton.

For the 1240-cm^{-1} band (Fig. 1 and Table 3), there is the frequency's blue shift in every group after irradiation by X ray. The most obvious blue shift of 8 cm^{-1} appears in the 8-Gy group. It is assumed that the obvious N−H distortion vibration and C=O, C=N stretch vibration happen in the Hela cells' protein of the 8-Gy group.

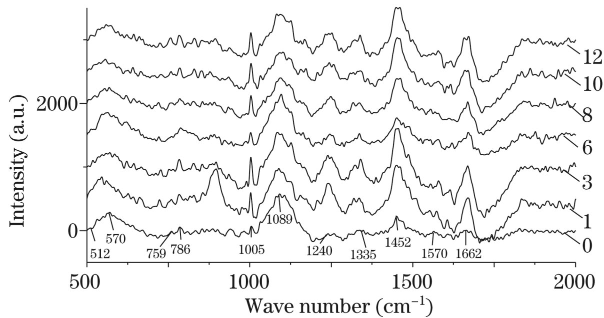

Fig. 1. Raman spectra of Hela cells irradiated by X rays of different doses and cultivated for 24 h sequentially.

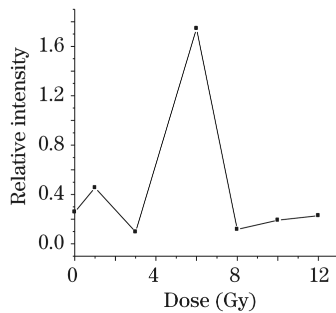

Fig. 2. Relative intensity of I_{1662}/I_{1005}.

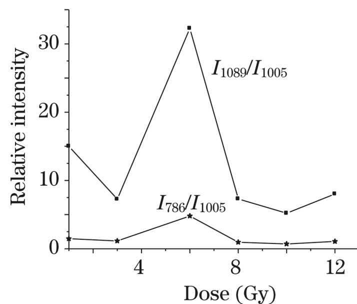

Fig. 3. Relative intensity of I_{786}/I_{1005} and I_{1089}/I_{1005}.

Table 1. Raman Spectrum Assignations of Hela Cells

Peak (cm^{-1})	Assignation
512	Stretch Vibration of S−S
570, 759, 1335	Vibration of Tryptophan Residue Indole Ring
786	Symmetry Stretch Vibration of O−P−O
885	Stretch Vibration of C−C Skeleton
1005	Phenylalanine Single-Base Replacing Phenyl Ring
1089	Symmetry Bent Stretch Vibration of O=P=O in DNA Skeleton
1157	Telescopic Mix of Carotenoid C−H and = C−C = in Carbohydrate
1240	Random Coil of Amide III
1452	Bent Vibration of CH_2 and CH_3
1570	Stretch Vibration of Carotenoid C=C in Carbohydrate
1662	Random Coil of Amide I

Compared with the control group's 1240 cm^{-1} band, some bands of the 12-Gy group moved to 1237 cm^{-1}. From this phenomenon, we assume that some random coil structures transform into β folding after irradiation by the high X ray dose.

The 1662-cm^{-1} band (Fig. 1 and Table 3) corresponded with amide I, and after irradiation by the 6-Gy X ray, it had a blue shift of 10 cm^{-1}. The 1672-cm^{-1} band corresponded with the β folding of amide I. Thus, this phenomenon showed that the random coil structure of the 6-Gy group transformed for β folding. From the intensity ratio of I_{1662}/I_{1005} (Fig. 2 and Table 2), the relative intensity of 6 Gy was the highest. Accordingly, the protein contents of the normal tissues were higher than that of the malignant tissues. We deduce that the 6-Gy dose merely supplies the energy which the conformation of the secondary structure needs, and the regular degree of protein can be better enhanced.

After irradiation by 1-Gy X ray, the 885-cm^{-1} band of the control group had a blue shift of 10 cm^{-1}, and an unusually prominent peak appeared. This phenomenon indicates that the new stretch vibration energy structure of C−C conforms with the 1-Gy group in comparison with the control group. But after irradiation by X ray of 6, 8, and 10 Gy, this kind of peak disappeared. This indicates that the X ray of 6, 8, and 10 Gy can destroy the old stretch vibration energy structure of the C−C skeleton.

The bands which belonged to the protein side-chain primarily came from aromatic amino acid protein residue[9,10]. In the Raman spectra of the control group, 570, 759, and 1335 cm^{-1} belonged to the vibration of the tryptophan residue indole ring[11] (Table 1). The 512-cm^{-1} band belonged to the stretch vibration of S−S[12] (Table 1), and the 1005-cm^{-1} band belonged to the substituent group which the phenylalanine single-base substituted with the phenyl ring (Table 1).

Because of 6-Gy X ray irradiation, the 759-cm^{-1} band disappeared, whichever appeared in the control group.

Table 2. Average Values ($\overline{x} \pm s$) of Intensity Ratios I_{1662}/I_{1005}, I_{786}/I_{1005}, and I_{1089}/I_{1005} of Every Group

Dose (Gy)	0	1	3	6	8	10	12
I_{1662}/I_{1005}	0.256±0.002	0.448±0.007	0.095±0.001	1.733±0.012	0.114±0.003	0.190±0.002	0.225±0.004
I_{786}/I_{1005}	1.054±0.016	1.447±0.020	1.134±0.010	4.780±0.020	0.948±0.004	0.705±0.003	1.057±0.012
I_{1089}/I_{1005}	12.195±0.018	14.988±0.002	7.263±0.010	32.260±0.006	7.309±0.005	5.204±0.007	8.021±0.003

Table 3. Average Values of the Bands ($\overline{x} \pm s$) and Their Assignations in Raman Spectra (cm^{-1})

Band	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
0 Gy	570.18±1.33	759.17±0.84	1004.59±0.28	1089.46±0.38	1240.65±1.23	1335.47±0.24	1452.10±1.08	1570.14±0.34	1662.35±0.30
1 Gy	562.16±0.33	757.78±1.23	1004.86±0.99	1087.72±1.05	1241.60±0.73	1336.10±0.95	1452.10±0.30	1572.67±0.22	1669.75±0.04
3 Gy	565.18±1.03	757.12±0.75	1004.48±0.41	1085.52±1.24	1241.54±0.51	1340.29±0.08	1452.10±0.37	1579.20±1.15	1668.30±0.68
6 Gy	563.03±1.63	*	1004.77±0.74	1095.21±1.30	1247.32±0.57	1336.10±0.53	1452.10±1.29	1576.00±0.39	1672.49±0.20
8 Gy	568.75±0.91	759.18±0.88	1004.64±0.42	1094.66±0.80	1248.75±0.90	1336.10±1.01	1459.43±0.22	1572.91±079	1667.78±0.08
10 Gy	556.12±0.27	759.31±0.88	1004.75±0.86	1098.16±0.39	1242.96±1.20	1339.14±0.42	1454.90±1.03	1578.11±0.80	1664.57±0.93
12 Gy	562.26±0.21	755.18±0.69	1004.63±1.06	1085.20±1.57	1247.28±1.10	1337.50±0.67	1454.79±0.13	1579.47±0.73	1669.72±0.17

*Peak disappeared.

The frequency deviation of the other groups were not obvious (Fig.1 and Table 3), indicating that the 6-Gy X ray just destroyed the molecular vibration energy structure, which produced the scattering light of 759 cm^{-1}. However, for the 570-cm^{-1} band (Fig. 1 and Table 3), every group had a red shift (8−14 cm^{-1}), and the changes of intensity were different. The 1335-cm^{-1} band (Fig. 1 and Table 3) in every group had a blue shift (2−6 cm^{-1}), and all the intensities increased. These phenomena indicate that compared with the control group, X ray irradiation only alters the molecular vibration energy structure that produces the scattering light of 570 and 1335 cm^{-1}, but cannot destroy them. Simultaneously, it is shown that the molecular vibration energy structures which produce the scattering light are different, although the bands at 570, 759, and 1335 cm^{-1} all belong to the vibration of the tryptophan residue indole ring.

Compared with the 512-cm^{-1} band (Fig. 1) of the control group, in the 12-Gy group it moved to 514 cm^{-1}, and its conformation was still distortion-distortion-distortion (g-g-g); the intensity of the 514-cm^{-1} band added 68.1%. Because the carbohydrate skeleton and S−S key both contribute to the 512-cm^{-1} band, and the conformation of the S−S key does not change, we assume that the damage to the S−S key of the protein side-chain is weaker than the carbohydrate skeleton.

The semi-width of 1005 cm^{-1} was narrow (Fig. 1 and Table 3). It had no frequency deviation after irradiation by X rays of different doses. This phenomenon shows that for the vibration band of the substituent group, conformation is not sensitive to X ray irradiation, which the phenylalanine single-base substitutes with the phenyl ring. If the protein covalent bond did not break, it would have been regarded as an internal standard. The result of this study conformed with the results of earlier studies. Thus, we calculated the relative intensity of some peak's intensities (1662, 786, 1089 cm^{-1}) divided by the 1005-cm^{-1} peak in every group.

DNA has always been regarded as an irradiation target. Direct and indirect radiation can both damage the structure of DNA. In the Raman spectra of control group, the bands which indicated the nucleic acid's characteristics were 786 and 1089 cm^{-1}; they came from two phosphoric acid skeletons of DNA, where 786 cm^{-1} corresponded to the symmetry stretch vibration of the phosphoric acid diester base (O−P−O), and 1089 cm^{-1} corresponded to the bent symmetry stretch vibration of the phosphoric acid diester key (O=P=O).

The 786-cm^{-1} band only had a blue shift of 3 cm^{-1} in the 6-Gy group (Fig. 1). It is shown that a non-hydrogen bond of phosphoric acid diester (O−P−O) increases after irradiation by 6-Gy X ray, and the double helix structure of DNA changes into disarray. The frequency deviation of 1089-cm^{-1} (Fig. 1 and Table 3) was erratic, it had a blue shift from 5 to 9 cm^{-1} in the groups of 6, 8, and 10 Gy, but had a red shift from 2 to 4 cm^{-1} in the groups of 1, 3, and 12 Gy. This phenomenon shows that the bent symmetry stretch vibration conformation of phosphoric acid diester key (O=P=O) in the nucleic acid is complex, and the duplet bond of the phosphoric acid diester key fractures.

The graph of I_{786}/I_{1005} (Fig. 3 and Table 2) is similar to that of I_{1089}/I_{1005} (Fig. 3 and Table 3), and the highest relative intensity appeared in the 6-Gy group. Compared with the respective control group, however, the proportion of every group changed differently, and showed that the content of the phosphoric acid diester base (O−P−O) and phosphoric acid diester key (O=P=O) in nucleic acid increased after irradiation by 6-Gy X ray, and the X ray of 6 Gy supplied enough binding energy and ionizing energy for O−P−O and O=P=O, thus the proportion became unbalanced, and the old nucleic acid structure of the Hela cells were destroyed, before finally perishing.

The cell membrane is another important irradiation target aside from DNA. The external membrane and intima are regarded as the biomembrane which includes protein, lipid, and glucide. The 1452-cm^{-1} band (Table 1) came from the vibration of lipids. The 1452-cm^{-1} band from the bent vibration of CH_2 and CH_3 in lipid, after irradiation by X ray with a high dose (8, 10, 12 Gy), had a blue shift, with the most obvious blue shift of 7 cm^{-1} appearing in the 8-Gy group (Fig. 1 and Table

3); the other groups did not have any frequency shift. It is related to the electronegative groups or the unsaturated groups connecting to the CH_3 group[13]. The X ray irradiation of the 8-Gy group severely affects the bent vibration energy structure of CH_2 and CH_3. This band is not sensitive to X ray irradiation of low dose. Compared with the control group, every group's intensity increased, proving the conclusion based on the frequency deviation. Bent vibration energy structures of CH_2 and CH_3 in the cells increased to meet the severe influence of irradiation. In addition, the 8-Gy group's intensity did not obviously increase, but showed part of the acyl chain skeleton breaking.

Although no peak appeared in the control group, the 1157-cm^{-1} band, which came from the telescopic mix of the carotenoid's C−H and =C−C= just appeared in the 1- and 10-Gy groups. This could have prompted the X ray irradiation of 1 and 10 Gy to alter the old carbohydrate structure, producing a new telescopic mix energy structure of carotenoid.

The 1570-cm^{-1} band came from the stretch vibration of the carotenoid's C=C in the carbohydrate (Table 1). Compared with the control group (Fig.1 and Table 3), every group had a blue shift ($2-9$ cm^{-1}), but the intensity all decreased, showing that X ray irradiation of different doses affected the stretch vibration structure of the carotenoid's C=C, hence making the C=C conjugated duplet bond oxidize, and the content of C=C finally decreased.

In conclusion, Raman spectra were used to study the structures and contents of Hela cells, while the Hela cells irradiated by X rays of different doses were cultivated for 24 h. The results showed the influence of 6- and 8-Gy X ray irradiation were most obvious, for example, in certain proteins, random coil structures transformed for β folding. The double helix structure of DNA changed into disarray, and part of acyl chain skeleton in lipids' CH_2 and CH_3 broke or connected, among others. We preliminarily conclude that for lowering the cancerization degree of Hela cells in the interval irradiation time of 24 h, X ray irradiation doses from 6 to 8 Gy are better than others. We plan to perform more experiments in the future to justify these results.

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