Characteristic Variation of α -Fetoprotein DNA Nanometer-Range Irradiated by Iodine-125

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Abstract

To obtain the characteristic variation of structure and functional groups of α -fetoprotein (AFP) DNA irradiated by iodine-125 (¹²⁵I), the AFP antisense oligonucleotide labeled with various radioactivity dose ¹²⁵I was mixed with the AFP DNA in a simulated polymerase chain reaction temperature condition. After the mixtures were irradiated by the ¹²⁵I from 2 to 72 hours, the mutation of the biogenic conformation and functional groups of the irradiated DNA were investigated using laser Raman spectroscopy. The shifted peak and the decreased intensity of the characteristic Raman spectra were found, which demonstrated that the structure of the phosphodiester linkage was broke, the pyridine and purine bases in DNA emerged and damaged. The model of gene conformation changed from form B to form C spectrum after the nanometer-range irradiation with ¹²⁵I from 2 to 24 hours. The damage of local pyridine and purine bases gradually increased along with the accumulation of irradiation, and the bases and ribosome were finally dissociated and stacked.

Key words: gene therapy, irradiation, radionuclide, Raman spectroscopy

Introduction

It is well established that the expression of α -fetoprotein (AFP) is closely related to the occurrence and development of some primary malignancies, such as hepatocellular carcinoma (HCC).^{1,2} The antisense oligonucleotide (asON) for the AFP gene is one of the efficient methods of gene therapy in HCC.² The results in our preliminary experiment demonstrated that the radioactive iodine-125 (¹²⁵I)-labeled asON of the AFP gene was more efficient to damage the AFP gene in HCC compared with only asON.^{3,4} This gene radiotherapy is mainly utilized the irradiation from radionuclide to damage the target gene.^{5,6} Auger electron emitter had been proposed as an attractive alternative to energetic emitters in gene radiotherapy.^{5,7} Among the Auger electron emitters, ¹²⁵I is one of the most promising options, because the ¹²⁵I Decay results in the emission of a large number of low-energy Auger electrons.^{6,8} However, the molecular mechanism of ¹²⁵I irradiation in gene radiotherapy is not understood. Some experiments' results indicated that the irradiation could result in destabilizing the DNA structure and decreasing the initial

level of DNA lesions.^{9,10} Because the DNA has an intricate helical and three-dimensional structure that keeps the DNA stabilization,^{11–13} the course of the structure of DNA broken by irradiation is complicated and vague.

Several lines of studies focused on the physical mechanisms responsible for radiotherapy with ¹²⁵I.^{6,14–16} Their results indicated that ¹²⁵I in close proximity to DNA reduced the Double-Strand Breaks per decay by a direct mechanism. The extent of the damaged DNA due to irradiation is dependent on the distance between the decaying ¹²⁵I atom and the cleaved deoxyribosyl unit.^{15,16} The Auger electrons emitted from ¹²⁵I deposit their all energy within several nanometers distance and their simultaneous action produces a considerable amount of damage to biomolecules, compared with that of an α particle.¹⁷ The Auger cascade would only produce disruption of ~5 base pairs on both sides of the decaying atom if the ¹²⁵I was affixed to a base that was incorporated into a duplex deoxyribonucleic oligonucleotide.¹⁶ A central decay of the Auger electron in DNA will give the highest absorbed dose, while at a distance of 3 nm, the absorbed dose will be only 10% of the maximum.^{6,16}

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However, the principle of DNA damaged by ^{125}I irradiation is not completely understood yet.^{7,17} There is little direct evidence on the structure of DNA destroyed by irradiation in the range of cubic nanometers, in which the experimental evidence of DNA damage was indirectly deduced from consequences detected by the biological technique, such as polymerase chain reaction, electrophoresis, and electron microscopy. Compared with these techniques, Raman spectroscopy is a versatile technique for investigating perturbations in the structure and conformation of nucleic acid *in situ* and without disturbance.^{18–20} It can determine detailed structural consequences of early events of irradiation damages on the molecular conformation and secondary structure of DNA, and probe the sequence-related structural differences among genomic DNA species.^{21–25} Therefore in our study, Raman spectroscopy was used to evaluate the damage of DNA by irradiation. In this experiment, the asON labeled with ^{125}I was mixed with the AFP DNA to induce the DNA strands damage *in situ*, and the structure of the DNA was evaluated with the Raman spectroscopy technique. Any change in the Raman spectra of the DNA under the irradiation will help to elucidate the molecular mechanism of target gene radiotherapy with irradiation of ^{125}I .

Materials and Methods

Samples labeling

The human AFP DNA and its asON (5'-AGC AGC TTG TTA AAT CAA CAT GCA-3') were purchased from the Biosciences and Technique Limited Company. The radioactive ^{125}I (Half life is 60.14 days) was purchased from the China Atom High Technique Limited Company. 5-iodine-deoxyurime (5-IdU) was obtained from the Sigma Limited Company. 5-IdU was labeled with radioactive iodine-125 (^{125}I -IdU) according to the methods reported by Schaffland et al.²⁶ Briefly, a mixture of 5-(tri-n-butylstannyl)-2'-deoxyuridine (Bu3SndUrd) (100 μg) in ethanol (20 μL), 0.1 M phosphate buffer pH 8 (70 μL), and sodium iodide was prepared. Then, the ^{125}I -IdU was linked with the AFP asON (^{125}I -AFPa) by means of terminal transferases. The radioactive-labeled rate was over 96% obtaining by HPLC purification and analysis.

Preparation of samples for Raman spectroscopy

The samples were divided into six different radioactivity groups, which were a1 (18.5 kBq), a2 (37 kBq), a3 (74 kBq), a4 (148 kBq), a5 (296 kBq) and blank controls (NC), respectively, and the a3 group was prepared with five same dose tubes for the experiment of series irradiation period (Table 1. Experimental design). The 10 μL 3.7 mM AFP DNA solutions were dripped to the polymerase chain reaction (PCR) tubes marked with numbers from No.1 to No.10, respectively.

Then, 18.5, 37, 74, 148, 296 kBq ^{125}I -AFPa were put into the tubes of No.1 to No. 5, respectively, and the tubes of No.6 to No.9 were added 74 kBq ^{125}I -AFPa each, whereas tube No.10 and all other tubes were added with deionized water to a 20- μL volume. The mixed complexes from tube No.1 to No.8 were processed in the way of simulated PCR condition (95°C 4 minutes, (95°C 20 seconds, 60°C 30 seconds) cycled 5 times, 4°C 10 minutes) to make ^{125}I -AFPa combine closely to the target DNA. The Raman spectra of the composites in tube No.1 to No.4 and the control tubes were recorded using a confocal Raman microscopy (LabRam INV) after the AFP DNA had been incubated with the ^{125}I -AFPa for 24 hours, the composites in tubes No.5 to No.8 were examined after 2, 12, 24, and 72 hours, respectively, and the complexes in tube No.9 and No.10 as controls were placed in 37°C for 24 hours.

Raman scan and data analysis

The structure, conformation, and the functional groups of the AFP DNA were evaluated with the Raman spectra recorded using the laser Raman microspectroscopy. The Raman spectra were excited with the 514.5-nm light of an argon laser. The effective spectral resolution was 1 cm^{-1} . Additional details about the recording settings and considerations were referred as the previous studies on living cells using the microspectroscopy.^{18,19,23} All the measurements were repeated in two independent experiments. To improve the signal to the noise ratio of the Raman spectra, up to 20 accumulated spectra, each taken for 15 seconds exposure time, were averaged over the spectral interval $400\text{--}1800\text{ cm}^{-1}$. The Raman spectra of the DNA solutions were normalized between 400 and 1800 cm^{-1} for preserving them from baseline deviations, and were subsequently corrected by subtracting the spectrum of a buffer solution prepared at identical experimental settings. The DNA Raman wave numbers, accurate to $\pm 1\text{ cm}^{-1}$, were calibrated using the 520-cm^{-1} band of a silicon chip before each sample recording. The method of semiquantitative calculation was applied to the acquisition of the relative spectral intensity (RIn), which is the intensity at a certain point in the spectrum (In) by subtracting the intensity of the base line (Ib), or $\text{RIn} = \text{In} - \text{Ib}$.

Results

No spectrum change was found in the samples in which ^{125}I -AFPa and AFP DNA just simply mixed for 24 hours. However, the samples in which the mixed complexes of ^{125}I -AFPa and AFP DNA were processed in the way of simulated PCR condition, there were obvious variation in the structure and conformation of the target DNA after the irradiation of ^{125}I in different intervals (2, 12, 24, and 72 hour). The Raman spectra of the irradiated DNA and control are displayed in Figure 1, respectively. The Raman characteristic frequencies, their relevant wave numbers, and assignments are

TABLE 1. LIST OF THE EXPERIMENTAL DESIGN OF GROUP, CONDITION, AND IRRADIATION TIME

No	1	2	3	4	5	6	7	8	9	10
Group	a1(18.5 kBq)	a2(37 kBq)	a3(74 kBq)	a4(148 kBq)	a5(296 kBq)	a3(74 kBq)	a3(74 kBq)	a3(74 kBq)	a3(74 kBq)	NC
Condition	PCR	PCR	PCR	PCR	PCR	PCR	PCR	PCR	no PCR	no PCR
Time	24 hours	24 hours	24 hours	24 hours	24 hours	2 hours	12 h	72 hours	24 hours	24 hours

PCR, polymerase chain reaction.

FIG. 1. The Raman spectra of the α -fetoprotein (AFP) DNA solutions after Auger electron irradiation closely emitted from iodine-125 in 0 (NC), 2, 12, 24, and 72 hours.

summarized in Table 2. The peak at 830 cm^{-1} shifted right to 832 cm^{-1} and its intensity became lower in Figure 1 after the irradiation with ^{125}I . The peak at 1089 cm^{-1} shifted blue by 6 cm^{-1} . A 1 cm^{-1} higher wave number of the band at 1743 cm^{-1} was also observed. The band at 784 cm^{-1} shifted

higher wave number by 3 cm^{-1} , its intensity decreased over 40 percent when the duration of irradiation time was more than 24 hours. In contrast, the intensity of the peak at 881 cm^{-1} increased with the irradiation time. A 3 cm^{-1} higher wave number of the peak at 1455 cm^{-1} was observed when the irradiation time was more than 24 hours, and the peak intensity was notably increased. Moreover, the intensity of the band at 926 cm^{-1} markedly increased when the irradiation time was 12 hours, but its peak disappeared when the irradiation time was 24 hours.

Transient alterations of the alkaline bases were observed in different intervals of Auger electron irradiation. The Raman peaks of the adenine bases at 701 , 1227 , 1308 , and 1612 cm^{-1} shifted to different extents. The Raman intensities of the peaks gradually decreased and even disappeared when the irradiation time was 72 hours. The Raman peaks of guanine at 659 , 1047 , and 1637 cm^{-1} had a mild higher shift, and the Raman intensity of the bands at 659 and 1637 cm^{-1} dramatically faded after 2 hours irradiation. The Raman peak of the bands for cytosine ring vibration at 444 and 1637 cm^{-1} shifted and decreased with the irradiation, and the Raman peak of the band at 784 cm^{-1} for the thymine ring vibration almost disappeared after 2 hours irradiation. These alterations of the Raman bands for bases revealed that there were perturbation in the guanine groups and disruption of the bases. However, the intensities of the bands at 881 , 1047 , and 1455 cm^{-1} increased as a consequence of irradiation. The trend of the relative spectral intensity (RIn) of the major bands on the irradiation time is illustrated in Figure 2. The figure display that the RIn of the Raman spectra fluctuate obviously in the period from 2 to 24 hours, but just change slightly after 24 hours. The intensities of the bands at 784 and 1743 cm^{-1} gradually decrease with irradiation time, whereas the intensities of the bands at 881 , 1047 , 1091 , and 1455 cm^{-1} increase.

The Raman spectra of the DNA complexes irradiated from a1 to a5 group with series concentrations of radioactivity ^{125}I

TABLE 2. RELEVANT WAVE NUMBERS AND ASSIGNMENTS MEASURED IN THE RAMAN SPECTRA OF α -FETOPROTEIN DNA SOLUTIONS WITH AUGER ELECTRON IRRADIATION IN SERIES PERIOD

Wave	2 hours	12 hours	24 hours	72 hours	Tentative assignments
444	448	444	445	446	Cytosine, & CH ₂
471	472	473	476	476	Thymine
659	660	660	657	657	Guanine
701	700	701	700	708	Adenine
784	781	786	788	787	Thymine, PO ₂
831	831	832	833	833	PO ₂ , B type
881	881	880	881	881	C type (d-p, bk)
926	927	928	930	929	Deoxyribose
1047	1049	1045	1045	1047	Guanine
1072	1075	1077	1080	1081	Glycine
1089	1092	1093	1095	1095	PO ₂ , bk
1191	1194	1191	1188	1188	C=N, Guanine
1227	1229	1227	1215	1217	Adenine
1308	1314	1308	1310	1299	Adenine
1455	1453	1455	1459	1458	Deoxyribose & CH ₂
1528	1526	1524	1533	1533	Cytosine, Guanine
1612	1611	1612	1613	1610	Adenine
1637	1637	1638	1630	1628	Guanine
1659	1672	1669	1667	1669	C=O, bk
1743	1744	1750	1743	1743	C-C twist

bk indicates a vibration of the DNA backbone. d-p indicates a vibration of the deoxyribose-phosphate moiety.

FIG. 2. The relative spectral intensity of the Raman bands of AFP DNA solutions after Auger electron irradiation in series period.

and controls (NC), respectively, are displayed in Figure 3 and Figure 4. Their Raman characteristic frequencies, relevant wave numbers, and assignments are summarized in Table 3. The Raman features shown in Figure 3 that can be assigned to this change are $701 \rightarrow 703 \text{ cm}^{-1}$ (adenine), $781 \rightarrow 778 \text{ cm}^{-1}$ (thymine), $1646 \rightarrow 1643 \text{ cm}^{-1}$ (the functional groups of thymine), $1271 \rightarrow 1275 \text{ cm}^{-1}$ (cytosine), $1424 \rightarrow 1422 \text{ cm}^{-1}$ (guanine), and the Raman intensities of the spectral peaks after irradiation became lower than those in control. The Raman spectra in Figure 3 demonstrate that the characteristic Raman vibration bands of DNA are extensive, and their peak intensities reduce obviously after the target DNA exposed to the irradiation from ^{125}I ($\sim 37 \text{ kBq}$ in $20\text{-}\mu\text{L}$ mixtures) for 24

hours. It was also found that the Raman band at 925 cm^{-1} higher wave number of 2 cm^{-1} after irradiated with $37 \text{ kBq } ^{125}\text{I}$ for 24 hours. The Raman band at 1094 cm^{-1} higher wave number of 2 cm^{-1} , and the Raman band at 1738 cm^{-1} shifted up to 1740 cm^{-1} . The Raman spectral intensity became weaker after the irradiation. However, the intensities of the Raman bands at 876 , 1088 , 1277 , and 1455 cm^{-1} gradually increased as the radioactive concentration on the DNA further increased over 74 kBq (Fig. 4). Several additional Raman bands at 646 and 1045 cm^{-1} (guanine), 783 cm^{-1} (thymidine) and 926 cm^{-1} (deoxyribose) are found in the Raman spectral curve a3, curve a4, and curve a5 in Figure 4, and their Raman peak intensities increased with increasing the concentration of ^{125}I . Figure 5 illustrates the variation of the spectral intensities of the bands at 646 , 783 , 876 , 926 , 1045 , 1090 , and 1278 cm^{-1} respectively, as functions of the concentration of ^{125}I . It clearly shows that the intensities gradually increase with the accumulation of the irradiation after the concentration of the ^{125}I is greater than 74 kBq .

Discussion

It is known that the irradiation biological effect has two mainly possibilities: a direct interaction or an indirect interaction.^{17,27} Because the carry range of Auger electrons is extreme short ($1\text{--}10$ nanometers), the spatial localization of the emitters relative to the targets may require a device with micron and, perhaps, an Angstrom resolution.^{5,27} A majority of research's results for Auger electrons' irradiation were the indirect interactions obtained from patient, animal, or cell culture studies.^{5,7,26,28} In our study, all the irradiation interactions of ^{125}I to DNA were investigated in a deionized microenvironment *in vitro* to decrease the indirect interactive irradiation effect. There was no spectrum change in the samples in which ^{125}I and AFP DNA was just simply mixed with Raman analysis, but obvious variation in the structure

FIG. 3. The Raman spectra of the samples in a1 (18.5 kBq), a2 (37 kBq) groups, and control irradiated by Auger electron for 24 hours.

FIG. 4. The Raman spectra of the samples in a3 (74 kBq), a4 (148 kBq), a5 (296 kBq) group irradiated with Auger electron for 24 hours.

and conformation of the target DNA in the mixed complexes of ^{125}I and AFP DNA processed in the way of simulated PCR condition.

In the previous studies, it was hypothesized that the Auger electron cascades from the incorporated iodine atoms

could destabilize the DNA structure and damage irreparably the DNA.^{7,26} These results were indirectly interpreted using biological medical techniques, such as comet assay, electrophoresis, and the polymerase chain reaction technique. It was difficult to obtain direct evidence of the alternative process of the DNA structure before the DNA was destroyed by the Auger electrons because of the low energy and consequent short range.^{17,27,29} Raman spectroscopy is a powerful technique capable of providing detailed information about the chemical nature of individual bonds.^{10,19,23,30} The shift of the Raman spectra peak can indicate the transformation of base groups and chemical bonds in DNA, and the change of Raman intensity can reveal the damage of base groups and chemical bonds in DNA.^{18,19,23}

In the current study, we had showed a lot of detailed alternative information of the conformation and secondary structure of DNA radiated by the direct interaction with the

TABLE 3. RELEVANT WAVE NUMBERS AND ASSIGNMENTS MEASURED IN THE RAMAN SPECTRA OF α -FETOPROTEIN DNA SOLUTIONS WITH AUGER ELECTRON IRRADIATION IN SERIES CONCENTRATIONS

Wave	18.5 kBq	37 kBq	74 kBq	148 kBq	296 kBq	Tentative assignments
445	449	442	443	442	445	Cytosine, & CH ₂
471	472	473	476	478	479	Thymine
646	650	652	650	647	648	Guanine
701	702	703	705	708	710	Adenine
781	778	776	778	781	783	Thymine, PO ₂
879	881	882	880	878	876	C type (d-p, bk)
925	927	928	930	925	926	Deoxyribose
1045	1049	1045	1045	1046	1047	Guanine
1071	1075	1077	1080	1081	1088	Glycine
1094	1092	1093	1095	1093	1090	PO ₂ , bk
1191	1192	1190	1191	1192	1194	C=N, Guanine
1271	1275	1276	1275	1274	1277	Cytosine
1388	1390	1391	1389	1390	1390	Adenine
1424	1422	1420	1423	1422	1425	Guanine
1453	1454	1455	1455	1455	1455	Deoxyribose & CH ₂
1526	1528	1527	1528	1527	1526	Cytosine, Guanine
1612	1611	1612	1613	1610	1611	Adenine
1646	1643	1641	1640	1638	1630	Thymine
1738	1740	1742	1741	1740	1743	C-C twist

bk indicates a vibration of the DNA backbone. d-p indicates a vibration of the deoxyribose-phosphate moiety.

FIG. 5. The relative spectral intensity of the Raman bands of AFP DNA solutions after Auger electron irradiation emitted from 18.5 to 296 kBq of iodine-125 in 20- μL mixtures.

Auger electron in different periods and in different concentrations of ^{125}I , using the analysis of the Raman spectra of DNA closely irradiated with ^{125}I in a microenvironment. The Raman spectra shift from 830 to 832 cm^{-1} , from 784 to 787 cm^{-1} , and from 1089 to 1095 cm^{-1} , were found in the Raman analysis of DNA radiated by the ^{125}I from 0 to 72 hours. These results indicated that the major B form backbone of DNA was altered by the irradiation with ^{125}I . Meanwhile, the higher wave number shift of the Raman band at 1743 cm^{-1} represented a C-C conformation change in nucleoside.^{19,20,31} On the other hand, the increase in the Raman peak intensity at 881 cm^{-1} demonstrated the alteration in the conformation of DNA and perturbations of the secondary backbone structure under the action of irradiation. A similar situation happened for the spectral peak at 1455 cm^{-1} , a 3 cm^{-1} higher wave number was observed, and the peak intensity was notably increased when the irradiation time was more than 24 hours. The Raman band at 926 cm^{-1} , which was assigned to the deoxyribose ring,^{19,22,31,32} was also found intensity increase by the irradiation for 12 hours, however, the peak disappeared after irradiation for 24 hours. These results revealed that the conformation and secondary structure of the deoxyribose was transformed by the irradiation of the Auger electron, and further destroyed when the irradiation was cumulated more enough. The alternations of the characteristic Raman spectrum of guanine, cytosine pyridine, and purine bases were also found, which revealed that there were perturbation and disruption of the bases.

We had observed the Raman spectra of DNA complexes irradiated by the ^{125}I in different concentrations from 18.5 to 296 kBq in 20- μL mixtures, some characteristic Raman bands of DNA found that their wave numbers were shifted, and their peak intensities reduced obviously after the target DNA exposed to the Auger electron emitted from ^{125}I (~37 kBq) for 24 hours. However, the Raman intensities of new bands at 646, 783, 876, 926, 1045, 1090, and 1278 cm^{-1} , which are the characteristic Raman bands of the damage of local DNA,^{10,19,23} gradually increased, while the ^{125}I dose increased, and several additional Raman bands at 646 and 1045 cm^{-1} (guanine), 783 cm^{-1} (thymidine), and 926 cm^{-1} (deoxyribose)^{19,32} become gradually strong (Fig. 5). These results indicated that the Auger electron from small dose of ^{125}I damaged the conformation of the DNA backbone and DNA local structure; Furthermore, this conformation resulted in the deviation and stack of the bases and ribosome, even though the damage of local DNA while the radioactive dose of the ^{125}I continually increased. We also found that the probe of AFP DNA was apart from the target DNA and their bases were stacked and accompany with the further increase of ^{125}I dose because the characteristic Raman bands of single-chain oligonucleotide at 877, 1088, 1277, and 1455 cm^{-1} were obviously increased.^{19,32}

The variations induced by irradiation on the target DNA were quite significant in our experiment. The above-mentioned detailed alternative information of Raman spectra provided the direct evidence that the nanometer-range irradiation with ^{125}I altered gradually the backbone structure and helical conformation of the target DNA, damaged local pyridine and purine bases. However, the irradiation effect of ^{125}I we observed was a short interaction effect in 72 hours.

Conclusions

In our experiment, the Raman spectra technique was applied to analyze the detailed alternative information of the conformation and secondary structure of DNA irradiated in different period and in series concentration of ^{125}I . The results of Raman spectra demonstrated that ^{125}I may alter gradually the backbone structure and helical conformation of the target DNA, damage local pyridine and purine bases and bring about the deviation and stack of the bases and ribosome. These irradiation damages of the conformation and structure of target DNA were directly detected *in situ* using the confocal laser Raman spectroscopy technique.

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Disclosure Statement

There are no financial conflicts.

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