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Abstract

A series of experiments was conducted to study the base composition of DNA in AVl2-induced tumor and host cells by paper chromatography, and it was found that DNA per cent. guanine-cystosine contents were around 42 % in both of them. The base composition of DNA of AV12 itself differs considerably from that of AVl2-induced tumor cells, while the DNA of tumor cells shows the property similar to that of host cell DNA. The genetical relationship among virus, host cells and tumor cells was discussed.

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BASE COMPOSITION OF DNA IN ADENOVIRUS-12-INDUCED TUMOR

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Whatever might be the mechanism through which cancer is induced by adenovirus type 12 (AV 12), it is easily conceivable that the nucleic acid, DNA in particular, is the substance that is directly associated with the specific properties of tumor cells. GREEN and PINA (1) reported that the guanine-cytosine contents of DNA in AV 12 (2) and AV 18 (3) differed from those in non-carcinogenic AV. It is the purpose of the present to describe the base composition of AV12-induced hamster tumor and host cell DNA.

MATERIALS AND METHODS

Virus and animals

0.1 ml of $10^{2.5}$ TCID50/0.1 ml Huie strain AV12 (supplied by Dr. YABE and cultured in HELA cells) was injected intraperitoneally into syrian hamsters less than 24 hours old. The tumor which developed within about two weeks in the peritoneal cavity were used for the experiment. Liver and brain of normal hamsters about one month old were used as controls.

Estimation of uucleic acid contents

Nucleic acids were extracted by the methods of SCHNEIDER (4) and OGUR-ROSEN (5). DNA contents were estimated by the diphenylamin reaction, and RNA contents by the orcinol reaction.

The preparation of DNA

For this purpose the SDS-phenol method as described by OHBA (6) was employed, and this purification process was repeated four times.

Electron microscope obseraations of DNA

The nucleic acid so prepared was dissolved in distilled water and negatively stained with 1 % phosphotungstic acid and observed with the electron microscope JEM-7.

Assay of the base composition of nucleic acids by paper chromatography

Ten milligrams of DNA were hydrolized with 0.15 ml 70 % perhydrochloric acid at 100°C for 60 min. Next, the paper chromatography was carried out with Toyo filter paper No. 51 using a mixture of methanol: conc. hydrochloric acid: water (70 : 20 : 10, v/v/v) as the solvent. For the detection of ultraviolet a filter

of author's own construction (7) was used. Spots were cut out and extracted with 0.1 N hydrochloric acid, and the blank was used as control. The ultraviolet absorbancy was determined by Hitachi spectrometer, and the base composition (mol %) was calculated by Bendich's extinction coefficient values (8): adenine = 12.6 (262.5 mμ), guanine = 11.1 (249 mμ), cytosine = 10.0 (276 mμ), thymine = 7.96 (265 mμ).

Estimation of thermal denaturation temperature (Tm)

This estimation was conducted with Hitachi spectrometer by the method of MARMUR and DOTY(9).

Determination of the sedimentation constant S of DNA

DNA was dissolved in 0.14 M NaCl/0.05 M acetate buffer solution and Hitachi analytical ultracentrifuge Model UCA-1A UV-B type was used for determination of the sedimentation constant.

RESULTS

Nucleic acid contents

By OGUR-ROSEN's method the following values were obtained:

RNA = 3.6 mg/g fresh tumor tissue

DNA = 14.2 mg/g fresh tumor tissue

By SCHNEIDER's method:

RNA = 3.8 mg/g fresh tumor tissue

DNA = 17.5 mg/g fresh tumor tisssue

It is obvious from these values that the ratio DNA/RNA in the animal tumor is considerably high (10).

Electron microscope observation of tumor DNA

The tumor DNA is observed as a linear unit of 20 Å in width and morphologically it does not differ appreciably from the DNA prepared with the liver and the brain of normal animals.

Analysis of the base composition of tumor DNA by paper chromatography

The tumor DNA was disintegrated into 4 bases, adenine, guanine, thymine and cytosine, and each was observed by the ultraviolet at the wave length of 2, 537 Å.

Respective four bases isolated from each spot were identified by their RF values and their maximum absorbancy at the UV spectra by the spectrometry, and on the basis of their absorbancy the base composition (mol %) was calculated. The base composition of tumor DNA was compared with that of the DNA prepared from the liver and the brain of normal hamsters (Table 1).

Although there can be seen some difference in the base composition between tumor DNA and the DNA of the liver and brain of normal hams-

Table 1 DNA Base Composition of Adenovirus-12-Induced Tumor
(Chemical analysis by paper chromatography)

Tissue	Guanine	Adenine	Cytosine	Thymine	% G-C
Adenovirus-12-Induced Tumor in Hamster	22.0	29.3	19.9	28.8	41.7
Normal Hamster Liver	25.0	28.5	17.2	28.7	42.1
Normal Hamster Brain	22.1	30.2	19.7	28.0	41.8

ter, % G-C in every case is about 42 %, showing little difference.

Thermal denaturation temperature of DNA

The denaturation temperature of DNA of the AV12-induced tumor, Tm (t)=86.4, and that of normal liver, Tm (1)=86.6, as calculated from the % G-C from the paper chromatography by the mutual relationship formula Tm=69.3+0.41 (G-C) of MARMUR and DOTY (9), both coincide practically with the actually measured values, revealing little difference between the DNA of tumor and that of the liver (Fig. 1).

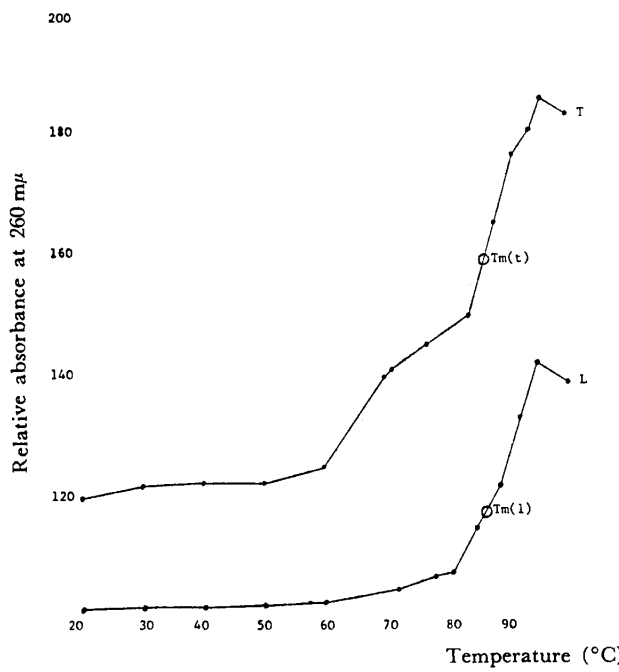

Fig. 1 The Hyperchromic Shift on Heating of DNA from Adenovirus-12-Induced Tumor (T) and Hamster Liver (L). The denaturation temperature (Tm) of DNA of the AV 12-induced tumor, Tm (t)=86.4 and that of normal liver, Tm (1)=86.6, as calculated from the %G-C from the paper chromatography by the mutual relationship formula Tm=69.3+0.41 (G-C).
Solvent: 0.15M NaCl+0.015M Na-Citrate PH=7.0

S value of DNA

S value of DNA from AV12-induced tumor is $S_{20}=28.0$, which is close to $S_{20}=29.3$ of normal hamster liver DNA, taking experimental error into consideration.

DISCUSSION

All the attempts made up to date at the isolation of infective virus from the AV12-induced tumor have failed. SMITH and MELNICK (11) have observed electron-microscopically virus-like particles in the filtered fluid of tumor, and OHMORI (12) has also observed virus-like particles in the thin section specimens of tumor. On the other hand, it is said that there exist type specific viral antigen (13, 14, 15), D antigen (16), transplantation antigen (17) and tumor antigens (18, 22) as immunologically detectable markers of the incomplete viral genomes. FUJINAGA and GREEN (19) have demonstrated the presence of viral genome in AV12-transformed hamster cell by hybridization measurements. The results of the present experiment indicate that the DNA % G-C content of the induced tumor is quite similar to that of the liver and brain of the host hamster, and likewise the denaturation temperature hardly differs from each other. Furthermore, as for S values of DNA as determined by analytical sedimentation, AV12-induced tumor DNA gives S value (28.0) close to that of normal hamster liver DNA (29.3), on the other hand S value of AV-2 is 32.0 and that of AV12 is 30.7 (20), being slightly lower than the S value of virus itself.

In view of the report by GREEN and PINA (20) in which they state that DNA % G-C of AV12 itself is 48 % and the denaturation temperature is 89°C, the property of the DNA of AV12 virus itself differs considerably from that of DNA in AV12-induced tumor cells and the base composition of tumor cell DNA is closer to that of the host cell DNA. Therefore, it is reasonable to assume that, when the target cells of host are transformed into tumor cells, they take up only a part of viral genome, i. e. the base pair change (21) in DNA as a genetical change, if any, is only partial to the host cell as a whole.

SUMMARY

A series of experiments was conducted to study the base composition of DNA in AV12-induced tumor and host cells by paper chromatography, and it was found that DNA per cent. guanine-cystosine contents were around 42 % in both of them. The base composition of DNA of AV12

itself differs considerably from that of AV12-induced tumor cells, while the DNA of tumor cells shows the property similar to that of host cell DNA. The genetical relationship among virus, host cells and tumor cells was discussed.

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