Mitochondrial DNA from hamster tumors induced by adenovirus type 12

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Mitochondrial DNA from hamster tumors induced by adenovirus type 12*

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Abstract

1. Mitochondria isolated from hamster tumors induced by adenovirus type 12 possessed circular DNA fibers. 2. The mean value of the length of the highest frequency group of the circular DNA molecules was 4.92 ± 0.38 µ. 3. Catenated dimer DNA molecules and small (less than 2 µ in length) circular DNA molecules were observed.

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Circular DNA molecules have been observed in mitochondria from various tissues (1—17). And many reports have revealed that mitochondrial DNA differs from nuclear DNA in many properties (18—29). Recently, investigations on the differences of mitochondrial DNAs between cancer cells and normal cells have been done in the field of carcinogenesis (6, 7, 11—17).

This report describes the electron microscopic observations on the mitochondrial DNA extracted from adenovirus Type 12-induced hamster tumors.

MATERIALS AND METHODS

Virus and animals: Adenovirus type 12 (Ad 12) was originally provided by Dr. KATSUO OGAWA of Department of Pathology, Okayama University Medical School. The stockvirus was propagated in our laboratory in HeLa cells. The infectivity titer of the virus was $10^{2.5} \text{TCID}_5/0.1$ ml.

0.05 ml of the stockvirus was injected intraperitoneally into syrian hamsters within 24 hours after birth. Forty-eight days after Ad 12 inoculation, tumors were excised, minced and very loosely homogenized. 0.5 ml of the cell suspension was subcutaneously inoculated into unconditioned hamsters of 45 to 60 days of age. Two weeks later, the hamsters were sacrificed, and the tumors were excised. Necrotic masses in the center of the tumors were elaborately excluded.

Isolation of mitochondria: Mitochondria were isolated according to the method of HOGEBOOM and SCHNEIDER (30). The crude mitochondrial fraction was suspended in 0.25M sucrose with 0.1mM EDTA and 2mM Tris buffer, pH 7.6 and layered on 0.34M sucrose. It was centrifuged at 700xg for 10 min. and approximately three fourths of the upper layer was carefully pipetted out. The mitochondrial fraction was washed four times with the sucrose solution (12,000 xg for 10 min.).

Isolation of DNA from mitochondria: DNA was isolated by MARMUR's method (31) with a slight modification. Since mitochondrial DNA fibers could not be spooled on a glass rod, the DNA-ethanol mixture was stored at $-20^\circ\text{C}$ overnight and it was centrifuged. The pellet was dissolved in dilute SSC. The absorption
spectrum of the suspension utilizing a Hitachi EPS-3T automatic spectrophotometer revealed that the wavelength at the maximal optical density was 260 m.$\mu$.

_Rotary shadowing and electron microscopy:_ Rotary shadowing of DNA fibers with platinum-palladium was performed following KLEINSCHMIDT and FREIFELDER's technique (32). The specimens were observed with a Hitachi HU-11C electron microscope. Contour length of DNA fibers was measured on electron micrographs.

**RESULTS**

In the electron microscopy of the specimens, considerably many, small linear DNA fragments (usually shorter than 2 $\mu$ in length) and numerous circular DNA fibers were observed. Long, linear fibers (longer than 5 $\mu$ in length) were far less frequently recognized. Circular DNA fibers were arranged in various forms; open, catenated and twisted. Highly twisted circles were occasionally observed. They were, however, excluded from measuring of the contour length, because of the difficulty in accurate measurement. Circular DNA molecules ranging in length from 4.0 to 5.5 $\mu$ occupied about 70 % of the total number of the circular-form DNA fibers, while those shorter than 2 $\mu$ in length amounted to approximately 18 %. The mean value of the length of the highest frequency group was 4.92±0.38 $\mu$.

![Histogram of the contour length distributions of circular mitochondrial DNA molecules isolated from hamster tumors induced by adenovirus type 12. Total number of circular DNA molecules: 57 Mean value of the length of the highest frequency group: 4.92±0.38 $\mu$](http://escholarship.lib.okayama-u.ac.jp/amo/vol24/iss6/1)

**DISCUSSION**

In mitochondrial DNA molecules from hamster tumors induced by Ad 12, the mean value of the length of the highest frequency group was
Fig. 2 An open circular mitochondrial DNA molecule isolated from hamster tumors induced by Ad 12. A few short linear DNA fibers are also observed.

Fig. 3 Two twisted circular mitochondrial DNA fibers
Fig. 4 Catenated circular mitochondrial DNA fibers. Two circular DNA fibers of the same length are catenated with each other.

Fig. 5 Small circular mitochondrial DNA fibers. (a) 0.3 μ (b) 0.4 μ (c) 0.9 μ (d) 1.0 μ (e) 1.3 μ

4.92 ± 0.38 μ. A report (10) described that the mean value of the length in mitochondrial DNA molecules from normal hamster liver was 5.13 ± 0.28 μ. Another recent report (11) showed similar data on the difference in length of mitochondrial DNA molecules between human normal liver and human hepatoma. The significance of the difference in length of circular DNA fibers between tumor and normal tissues is unknown.

The presence of small circular DNA in tumor cell mitochondria aroused the interest of many workers because small circular DNA viruses were highly oncogenic in some animals and cultured cells. However,
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BENJAMIN (33) showed the absence of homology between DNA from small circular DNA viruses and mitochondrial DNA from virus-induced tumors. It still remains unknown whether the difference in length of circular DNA fibers between normal and tumor tissue mitochondria is of any significance in oncogenicity.

The most probable explanation on short linear DNA fibers is that some mitochondrial DNA molecules had been broken into small pieces through the procedure of DNA extraction. A small number of the linear DNA fibers might be originally present in mitochondria. As to the long linear fibers, there may have been a little nuclear contamination in the mitochondrial fraction.

SUMMARY

1. Mitochondria isolated from hamster tumors induced by adenovirus type 12 possessed circular DNA fibers.
2. The mean value of the length of the highest frequency group of the circular DNA molecules was $4.92 \pm 0.38 \mu$.
3. Catenated dimer DNA molecules and small (less than $2 \mu$ in length) circular DNA molecules were observed.

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REFERENCES

7. CLAYTON, D. A. and VINOGRAD, J.: Circular dimer and catenate forms of mito-


30. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E.: Cytochemical studies of mammalian tissues. 1. Isolation of intact mitochondria from rat liver: some biochemical
Mitochondrial and from Hamster Tumors Induced by Adenovirus Type 12

properties of mitochondria and submicroscopic particulate material. J. Biol. Chem. 172, 619, 1943


33. BENJAMIN, T. L.: Absence of homology between polyoma or SV40 viral DNA and mitochondrial DNA from virus-induced tumors. Virology 36, 685, 1968