Adenovirus-induced Chromosome Aberrations in Human Cells

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SUMMARY

Infection of primary human embryo kidney cells with adenovirus types 2, 7, 12, 18 and 31, at a multiplicity of infection of 2, resulted in the induction of chromosome aberrations in metaphases examined 24 hr after infection. The aberrations induced by types 2 and 7 were randomly distributed over the karyotype, but types 12 and 31, and to a lesser extent type 18, all induced non-random effects. Both type 12 and type 31 produced chromatid and isochromatid gaps and breaks at a specific site on chromosome no. 17.

INTRODUCTION

Adenoviruses have been shown to cause chromosome changes in cells which are permissive for the virus and also in non-permissive cells. Chromatid gaps and breaks, fragmentation and chromosome rearrangements have been reported in hamster cells following infection with adenovirus types 2, 4, 7, 12 and 18 (Stich, Van Hoosier & Trentin, 1964; Stich & Yohn, 1965; MacKinnon et al. 1966). Huang (1967) found a non-random occurrence of aberrations in Rattus (Mastomys) natalensis cells following infection with adenovirus type 12. Adenovirus types 12 and 18 have also been shown to induce cytogenetic changes in a continuous line of human cells, Hep 2 (Stich, Avila & Yohn, 1968).

Adenovirus type 12 infection of human leucocytes, which are non-permissive for this virus, was reported by Nicholls et al. (1968) to result in a random distribution of chromosome damage, but the same serotype has been shown by zur Hausen (1967) and McDougall (1970) to induce non-random chromosome aberrations in human epithelial and fibroblastic cells.

Piña & Green (1969) described experiments in the human KB cell line in which thymidine kinase activity was increased two- to threefold in cells infected with adenovirus types 12 and 31, but by only 20% in cells infected with type 2. As there is some cytogenetic evidence from cell hybridisation studies that a locus for this enzyme may be situated on chromosome no. 17 (Weiss & Green, 1967; Migeon & Miller, 1968), and as this chromosome is affected at high frequency by type 12, the interaction of type 31 with the chromosomes of human cells was examined to determine whether or not any specific damage was caused to chromosome 17. The results obtained, together with results from experiments with types 2, 7 and 18 in primary human cells are presented in this report.

METHODS

Viruses. Adenovirus types 2, 7, 12 and 18 were supplied by Dr H. G. Pereira (National Institute of Medical Research, Mill Hill, London, N.W. 9) and type 31 by Dr M. S. Pereira (Central Public Health Laboratory, Colindale, London, N.W. 9). These strains were passaged three times in human embryo kidney (HEK) cells and were then purified from fluorocarbon extracts of infected cells by density gradient centrifugation in caesium chloride, using the
method of Russell, Valentine & Pereira (1967). Infectivity titrations (TCD 50) were carried out in HEK cells and end points calculated by the method of Reed & Muench (1938).

**Cells and media.** HEK cells were obtained by trypsinization of kidneys from embryos removed by therapeutic abortion. The cells were grown in 9 cm. plastic Petri dishes (Sterilin) incubated at 37° in an atmosphere of 5 % CO₂ in air. The medium used for cell cultivation and for experiments was Ham's F10 (Ham, 1963) supplemented with 10 % tryptose phosphate broth and 20 % foetal calf serum (FCS, Flow Laboratories).

**Cytogenetic studies.** HEK cells were seeded in 5 cm. plastic Petri dishes at 5 × 10⁵ cells/dish in 5 ml. of medium. After 48 hr incubation at 37° the cells were washed twice with phosphate buffered saline and infected by adsorbing 10⁶-3 TCD 50 of adenovirus in 0.2 ml. for 3 hr at 37°. After the adsorption period the cells were washed with phosphate buffered saline, re-fed with 5 ml. of medium and incubated at 37° in 5 % CO₂/air for a total period of 21 hr, including the 3 hr period during which Colcemid was present. The method for making chromosome preparations was as previously described (McDougall, 1970).

<table>
<thead>
<tr>
<th>Chromosome group</th>
<th>Adenovirus serotype</th>
<th>Uninfected control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 2</td>
<td>Type 7</td>
</tr>
<tr>
<td>A 1</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>A 2</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>A 3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>B 4/5</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>C 6-12+X</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>D 13-15</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>E 16</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E 17</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>E 18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>F 19/20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>G 21/22</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>78</td>
</tr>
</tbody>
</table>

| No. cells with damage | 48 | 46 | 56 | 39 | 45 | 5 |
| No. cells examined | 100 | 100 | 100 | 100 | 100 | 100 |

Results are totals from experiments in two batches of HEK cells. Chromosome groups in accordance with the Denver classification (1960).

**RESULTS**

The results of chromosome studies in two batches of HEK cells are shown in Table 1. There was a high incidence of chromosome aberrations following infection with all of the adenovirus serotypes tested at an m.o.i. = 2 (Table 1). From the histograms in Fig. 1, in which the distributions of virus-induced aberrations are compared with the distribution of chromosomal material in the human karyotype, it can be seen that types 2 and 7 induce random damage, that type 18 induces more damage in chromosome no. 17 than is compatible with a random distribution and that types 12 and 31 have a high degree of specificity for chromosome no. 17. In the case of type 12 there is also a non-random effect on chromosome no. 1, as previously reported (zur Hausen, 1967).
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The aberration which is induced in chromosome no. 17 by types 12 and 31 is consistently found to be a chromatid or chromosome gap or break proximal to the mid-point of the long arm of this autosome (Fig. 2). A karyotype of an uninfected cell is shown in Fig. 3. A secondary constriction has been reported to occur at this position (Ferguson-Smith, 1962).

(Pahner & Funderburk, 1965), but is rarely seen in normal human cells. Chromatid and chromosome gaps were occasionally induced by type 31 at the same locus on the no. 1 chromosome as that affected by type 12, but there was no increase in the incidence above that expected in a random distribution. The aberrations found in the no. 17 chromosome after infection by type 18 were in two positions. In some cells the damage was identical to that found with types 12 and 31 but in the majority of cells the chromatid gap was in a near terminal position. When cells were infected at m.o.i. > 2, using any of the viruses examined, the number of cells in mitosis at 24 hr after infection was reduced. Extensive chromosome damage (Fig. 4) could be found in metaphases from cultures with high virus input, and the damage was distributed in a random manner (Fig. 5). However, when the aberrations in the

Fig. 1. Distribution of virus-induced chromosome aberrations compared with distribution of chromosomal DNA (shaded histogram). The DNA histogram is based on the relative lengths of chromosomes classified in groups (Denver Conference, 1960). Results based on 100 cells/sample.
extensively damaged cells from cultures infected with types 12 and 31 were further analysed by examining the localization of gaps and breaks in regions of the chromosomes, it was found that the distribution was non-random in chromosome no. 17 (Fig. 6). This result emphasizes the necessity for (i) accurately estimated virus input values during studies of virus/chromosome interactions and (ii) further analysis of chromosome sites affected in extensively damaged cells.

Fig. 2. Chromosomes from an adenovirus type 31 infected HEK cell (m.o.i. = 2). One no. 17 chromosome has a chromatid gap and the other a chromatid gap and a chromatid break. There is also a chromatid gap in the long arm of a no. 2 chromosome.
Fig. 3. Normal human cell with the chromosomes arranged according to the Denver classification.

**DISCUSSION**

The consistency with which adenovirus type 12 causes gaps and breaks at a site on the long arm of chromosome no. 17 is a remarkable and unexplained phenomenon. The presence of a secondary constriction at this site has been observed on rare occasions in
normal human cells and has also been reported in a pseudodiploid permanent human cell line (Miles & O'Neill, 1966) and in human cells infected with the oncogenic simian virus SV40 (Moorhead & Saksela, 1963). If the enhancement of a pre-existing secondary constriction were a general consequence of virus infection or, in particular, of adenovirus infection then this enhancement should be seen in metaphases from cultures infected with

Fig. 4. Chromosomes from an adenovirus type 31 infected HEK cell (m.o.i. = 50). There are numerous chromatid gaps including an isochromatid gap on a no. 17 chromosome.
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any serotype. However, the finding that adenovirus types 2 and 7 can induce chromosome aberrations in primary human cells but do not have a specific effect on chromosome no. 17 is not consistent with that hypothesis.

Adenoviruses were classified by Huebner (1967) into three groups based on the degree of oncogenicity in hamsters. In this classification types 12, 18 and 31 are in the highly oncogenic group, type 7 is moderately oncogenic and type 2 non-oncogenic. Freeman et al. (1967) and McAllister et al. (1969) have shown that although the oncogenicity classification based on

![Histogram of Total DNA (%): Chromosome groups A, B, C, D, E, F, G, Y]

Fig. 5. Effect of virus input on the distribution of chromosome aberrations. HEK cells were infected with adenovirus type 31 and chromosome preparations made after 24 hr at 37 °C. Fifty cells with chromosome aberrations were recorded for each sample. Adenovirus at m.o.i. = 50 induced 13.7 aberrations/cell and at m.o.i. = 2 induced 2.4 aberrations/cell.

![Histogram of Aberrations (%): m.o.i. 50 and m.o.i. 2]

Fig. 6. Localization of damage induced in short and long arms of chromosomes 17 and 18 by adenovirus type 31 at high multiplicity of infection (m.o.i. = 50).

hamsters is valid; viruses in Huebner’s non-oncogenic group do have the capacity to induce cellular transformation in vitro. This evidence, together with that reported for adenoviruses from species other than man (Sarma, Huebner & Lane, 1965; Darbyshire, 1966; Hull et al.
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1965), indicates that the ability to induce cellular changes is a property common to all members of this virus group.

It is of interest that two of the highly oncogenic adenoviruses (types 12 and 31) both exhibit non-random effects on human chromosomes and induce specific damage on the same chromosome. The third member of this highly oncogenic group (type 18) has also been shown to exhibit some specificity for this chromosome, although not always at the same locus.

The relationship between chromosome changes and neoplasia is still a topic for investigation. Many agents other than viruses – for example, X-rays (Evans, 1967) and carcinogenic chemicals (Kato, 1968) – which damage chromosomes can also induce tumours. Many tumour cells exhibit cytogenetic changes when compared with normal cells, but there is as yet no evidence that the chromosome changes are causal in tumour development. Cellular changes similar to those seen early in virus-induced transformation have been reported only rarely in human cells infected in vitro with adenovirus type 12 and have not resulted in the establishment of permanent cell lines (Todaro & Aaronson, 1968; Shevliaghyn & Karazas, 1970). Attempts at finding evidence of adenovirus involvement in the aetiology of human tumours have also been unsuccessful (Sabin, 1968).

The three highly oncogenic serotypes have a number of characteristics in common apart from the ability to induce tumours in hamsters at high frequency, e.g. H.A. subgroup, G+C ratio, buoyant density and molecular weight (Schlesinger, 1969). From the results presented here another shared characteristic is the induction of specific damage in a human autosome. It has been shown by Kit, Nakajima & Dubbs (1970) that human adenoviruses do not code for a new virus-specific thymidine kinase, and therefore that the enhanced levels of this enzyme found in adenovirus-infected cells are coded for by the host cell genome. The fact that the specific chromosome aberration in chromosome no. 17 is induced at high frequency by types 12 and 31, taken in conjunction with the previously mentioned experiments on thymidine kinase induction (Piña & Green, 1969) and the cell hybridization studies (Weiss & Green, 1967; Migeon & Miller, 1968), may indicate that the gap seen in this chromosome at metaphase represents a virus-induced transcription of uncondensed host DNA with a virus-induced enzyme as the final product.

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REFERENCES

HUANG, C. C. (1967). Induction of a high incidence of damage to the X chromosomes of Rattus (Mastomys) natalensis by base analogues, viruses and carcinogens. Chromosoma (Berlin) 23, 162.
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