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Tumor induction by human adenovirus type 12 in hamsters: loss of the viral genome from adenovirus type 12-induced tumor cells is compatible with tumor formation

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The patterns of integration of adenovirus type 12 (Ad12) DNA in 39 virus-induced hamster tumors were determined. Both the amount of Ad12 DNA persisting and the apparent sites of insertion differed from tumor to tumor. In 30 tumors, the intact Ad12 genome persisted in colinear arrangement and in multiple copies. In nine tumors, only the left- or the left- and right-hand parts of the Ad12 genome persisted in the tumor cells. In three other cell lines the Ad12 genomes were lost completely during continuous passage in culture. A shift from epithelioid to fibroblastic morphology correlated with loss of the Ad12 genomes. The cell line H1111(1) derived from an Ad12-induced tumor had lost all viral DNA by the thirteenth subpassage, but was still oncogenic when reinjected into animals. This finding raises the question, to what extent persistence of the Ad12 genome is essential for the oncogenic phenotype. Tumor cells could be detected histologically inside local lymphatic vessels. In those experiments in which Ad12 preparations were used which contained sizeable proportions of the symmetric recombinant between Ad12 and KB cellular DNA (Deuring *et al.*, 1981), tumors were observed in the nuchal region of the animals.

Key words: local metastases/symmetric recombinant/integration patterns/change in morphology

Introduction

The injection of purified human adenovirus type 12 (Ad12) into newborn Syrian hamsters *(Mesocricetus aureatus)* leads to the induction of undifferentiated sarcomas at the site of injection (Trentin *et al.*, 1962; Huebner *et al.*, 1962). Distant metastases of these tumors have not been described.

Recently, the patterns of integration and of methylation of Ad12 DNA in 11 tumors and in cell lines established from them have been determined (Kuhlmann and Doerfler, 1982). In the tumor cells Ad12 DNA persists in multiple copies, and the bulk of the Ad12 DNA molecules is integrated intact and colinear with the virion DNA. The integrated viral genomes are strikingly undermethylated. Upon explantation and/or cloning of cells from the tumors, the integration patterns can change, and an increase in the extent of DNA methylation is observed (Kuhlmann and Doerfler, 1982). One interpretation is that Ad12-induced tumors originate from one or a limited number of clones of transformed cells and that on explantation and/or on cloning, one of these original clones is selected. It is also conceivable that initially all cells in a tumor have similar or identical integration patterns, and that the variability results from rearrangements of integrated viral DNA and adjacent cellular DNA sequences during or after integration. Moreover, the extent of DNA methylation seems to depend on cell culture conditions.

We have now compared the patterns of integration in several different Ad12-induced tumors and examined how they change with variations in culture conditions. We have also tried to correlate changes in integration patterns of viral DNA with alterations in the biological properties of tumor cells in culture. Adenovirus-transformed and tumor cells can serve as tools for studies on the differential expression of integrated viral DNA.

The patterns of Ad12 DNA integration have been determined in the DNA from 39 different tumors. As judged from the locations of restriction sites, the sites of linkage between cellular and viral DNA in these tumors appeared to have quite different macroenvironments. Nine tumors had lost considerable amounts of the viral DNA. When tumor cell lines or cloned cell lines were established from the tumors, in general the patterns of viral DNA integration did not change. In three instances, all of the viral DNA was lost from the cells. The cell lines retaining Ad12 DNA kept an epithelioid morphology, whereas cell lines having lost all Ad12 DNA sequences reverted to fibroblastic morphology. Some of these fibroblastic revertants were derived from cloned populations of epithelioid cells still carrying the Ad12 genome. The fibroblastic revertants still proved oncogenic in susceptible hamsters; the apparent loss of the Ad12 genome did not result in loss of the oncogenic phenotype.

Results

Induction of tumors by Ad12 in hamsters

Newborn hamsters were injected with Ad12 or a mixture of Ad12 and particles containing a symmetric recombinant (SYREC) between Ad12 and KB cellular DNA (Deuring *et al.*, 1981). The terminal 750–1150 base pairs from the left end of Ad12 DNA constitute the termini of SYREC. The remainder of the molecule, which is indistinguishable in length from authentic Ad12 DNA, is made up of human KB cell DNA. It cannot be ruled out that most Ad12 preparations contain minute amounts of SYREC particles.

Table I summarizes the results. Of the 83 animals injected, 42 (51%) survived and 30 (71%) of the survivors developed tumors. Of these 30 animals, 18 had received Ad12 preparations containing sizeable proportions of SYREC particles. Among these 18 animals, 7 (39%) developed tumors at the site of injection and/or in the nuchal region, whereas 11 hamsters (61%) exhibited tumors at the site of injection only. The 12 hamsters that were injected with Ad12 lacking considerable amounts of SYREC showed tumors exclusively at the site of injection. Hamsters injected with phosphate-buffered saline developed no tumors for nine months.

Biological properties of Ad12-induced tumors

The tumors were rich in small round, large round, or oval cells and were well vascularized; sometimes tumor cells were

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Table I. Induction of tumors by Ad12 in hamsters

1 Animal no.[a]	2 Sex[b]	3 Virus preparation[c]	4 Localization of tumor[d]	5 Number of tumors[e]	6 Period of latency (days)[f]	7 Tumor growth (days)[g]	8 Serum of animal precipitated T antigen[h]	9 Ad12 DNA in tumor cells (copies/cell)[i]	10 Original cell morphology	11 Shift to fibroblastic morphology upon subcultivation
T211	m	1	I	2	43	18	+	N.D.[j]	–	–
T212	m	1	I	1	55	43	+	~15	epithelioid	N.D.
T213	m	1	I	1	330	29	–	1–2	fibroblastic	
T311	f	2	I	1	43	17	+	N.D.	–	–
T312	f	2	I + N	3	50	28	N.D.	N.D.	–	–
T313	f	2	N	1	56	23	+	~30	epithelioid	+
T314	f	2	I	1	65	65	+	2–3	epithelioid	+
T315	f	2	I + N	2	73	16	+	4–5	epithelioid	N.D.
T316	m	2	N	1	91	59	+	6–7	fibroblastic	
T411	f	1	I	3	42	38	+	1, 2, 3	epithelioid	N.D.
T412	f	1	I	1	49	29	+	1–2	epithelioid	+
T413	f	1	I	1	49	30	+	8–9	epithelioid	N.D.
T414	f	1	I	1	76	92	+	2–3	fibroblastic	
T511	f	1	I	1	48	37	+	3–4	epithelioid	N.D.
T512	f	1	I	1	63	22	+	1–2	epithelioid	N.D.
T1111	m	1	I	2	38	34	–	10–11	epithelioid	+
T1112	f	1	I	2	42	32	+	8–9	epithelioid	+
T1113	m	1	I	2	59	29	+	3–4	epithelioid	N.D.
T1211	m	2	I	1	36	14	–	1–2	epithelioid	N.D.
T1212	f	2	I + N	2	42	8	–	3–4, 8–9	epithelioid	N.D.
T1213	f	2	I	1	47	23	+	~30	epithelioid	+
T1214	f	2	N	1	47	18	N.D.	2–3	epithelioid	N.D.
T1215	m	2	I	1	135	21	+	2–3	fibroblastic	
T1216	m	2	I	3	150	56	–	2–3	fibroblastic	
T1311	m	2	I	1	34	16	+	2–3	epithelioid	N.D.
T1312	f	2	I	2	45	18	N.D.	N.D.	–	–
T1313	m	2	I	1	51	22	+	4–5	epithelioid	+
T1314	f	2	I	3	54	32	+	1, 30, 35	epithelioid	N.D.
T1315	f	2	I	4	65	33	+	6–7	T(2) fibrobl. T(4) epithel.	–
T1316	m	2	N	1	65	21	–	1–2	epithelioid	N.D.

[a]The first digit (from 11 on, the first two digits) indicates the number of the pregnant hamster, the second (third) digit the litter number, and the last digit the individual tumor-bearing animal.

[b]m = male; f = female

[c]1: Ad12 preparations without considerable contaminations with SYREC particles.
2: Ad12 preparations containing SYREC particles.

[d]I: Site of injection.
N: Nuchal region.

[e]In some of the animals more than one tumor was observed. Some of these tumors were analyzed separately; e.g., three tumors in hamster 1314 were analyzed for the amount of Ad12 DNA persisting. In some animals (413, 1111, 1112, 1214) several separate tumors could be distinguished at early stages of tumor development. Later, these tumors coalesced.

[f]The time at which the tumors were first detected by palpation.

[g]Time of observation after first appearance of tumors.

[h]Sera from tumor-bearing animals were tested for their capacity to precipitate the 60 K T-antigen from extracts of the Ad12-transformed hamster cell line HA12/7 (Stabel *et al.*, 1980). Technical details were described elsewhere (Achten and Doerfler, 1982).

[i]The number of Ad12 DNA copies persisting per tumor cell was measured as described in **Materials and methods** (Stabel *et al.*, 1980). In brief, tumor cell DNA was cleaved with the EcoRI restriction endonuclease, the fragments were separated by electrophoresis on horizontal agarose slab gels, denatured and transferred to nitrocellulose filters by the Southern technique. On the same gels, EcoRI-cut Ad12 DNA in amounts corresponding to 1, 5, 10, 20, and 30 genome equivalents were coelectrophoresed and transferred. The amounts of Ad12 DNA used in this series of reconstitution experiments were based on a total of 10 μg of cellular DNA per gel slot. After completion of transfers, viral DNA sequences were localized by hybridization with ^{32}P-labeled nick-translated Ad12 DNA followed by autoradiography. Autoradiographic intensities were compared for the internal fragments B, D, and E of Ad12 DNA between cellular and authentic Ad12 DNA in the reconstitution series. A scanning microphotometer was used in the analysis. The internal EcoRI fragments of Ad12 DNA had to be used, since transfer in the higher mol. wt. off-size positions of the terminal fragments might not always be complete. In some cases (T213, T1211, T1216, T1316, T1314/1, T1314/2) only the EcoRI C fragment persisted.

[j]N.D. = not determined

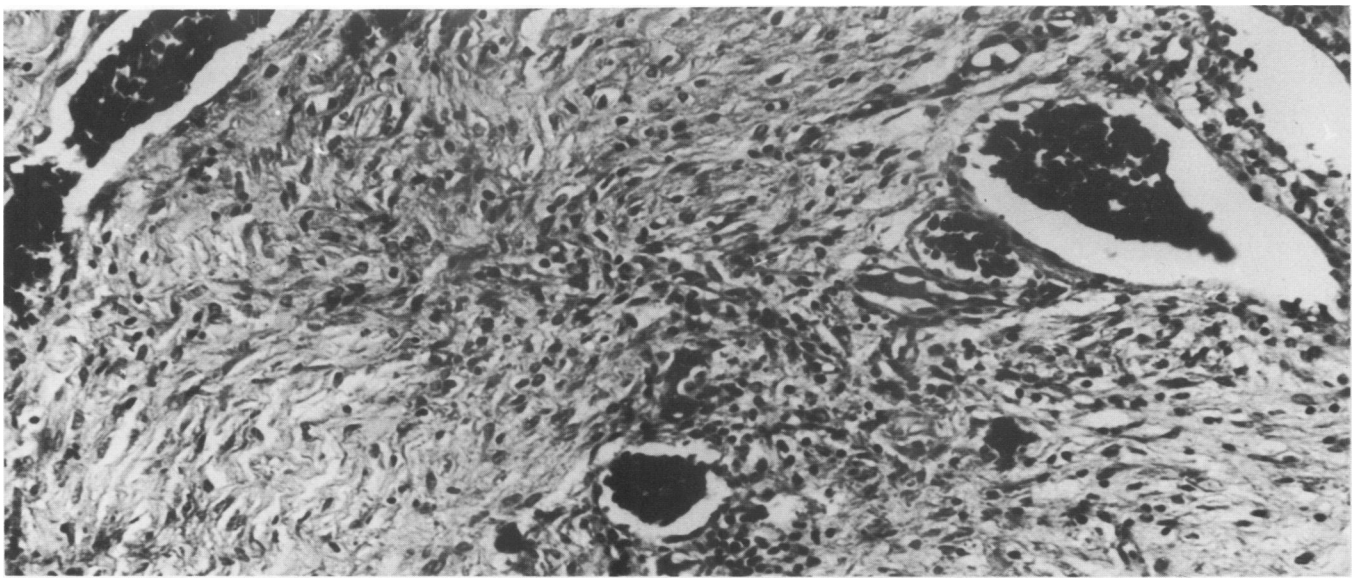


Fig. 1. Thin section of connective tissue with lymphatic vessels. The section was stained with hematoxylin-eosin and was derived from the animal bearing tumor T212. Magnification x 500.

arranged rosette-like around blood vessels. Most tumors were hemorrhagic and necrotic. In several instances, tumor cell masses were found inside lymphatic vessels within the tumor capsule (Figure 1). Metastases in the large parenchymal organs were never observed. Frequently the morphology of tumor cells changed upon explantation and subcultivation. Upon reimplantation of cultured cells into newborn animals and reexplantation into culture, the morphology of the cells remained unaltered and was very similar or identical to that of the injected cells. Cells reexplanted from these reimplantation tumors turned fibroblastic in culture.

The development of tumor mass was followed from first observation of the tumor to exstirpation as indicated in Table I (column 7). The largest diameters of the tumors were taken as approximate measures of tumor growth. In most cases, the rate of enlargement was rapid; tumors reached diameters of 4 cm by 30–40 days after first observation. Tumors developing in the nuchal region of the animal injected with Ad12 preparations which contained SYREC particles (Deuring *et al.*, 1981) expanded more rapidly than tumors at the sites of injection.

Sera from most of the tumor-bearing animals were tested for their ability to precipitate a 60 K protein from extracts of the Ad12-transformed hamster cell line HA12/7 (Table I, column 8). Other Ad12-specific proteins were occasionally also precipitated. Among the 27 sera tested, 21 (77.8%) precipitated the 60 K polypeptide, sera from six animals did not. Four sera in the latter category were from animals whose tumors (T213, T1211, T1216, T1316) contained exclusively Ad12 DNA fragments covalently linked to cellular DNA.

Cell lines were established from 14 different Ad12-induced tumors (Kuhlmann and Doerfler, 1982). The morphology of the cells in first passage derived from 39 tumors is recorded in Table I (column 10). Upon prolonged passage in culture, cells remained epithelioid or changed to fibroblastic morphology (Table I, column 11). In several instances, e.g., line H1111(1), the cells that had reverted to fibroblastic morphology had lost all copies of Ad12 DNA, whereas epithelioid cells usually retained the amount of Ad12 DNA found in the original tumor (see below).

The amount of Ad12 DNA persisting in tumor cells

The amounts of Ad12 DNA persisting in the cells of each tumor investigated were determined as previously described (Stabel *et al.*, 1980) and as outlined in the legend to Table I (Footnote i). In different tumors the number of copies of Ad12 DNA per cell ranged from 1–2 to ~30–35 (Table I, column 9). The amount of viral DNA persisting could best be estimated from the amounts of the internal fragments of the Ad12 genome (EcoRI fragments B, D, and E). The terminal EcoRI fragments A and C were joined to cellular sequences and hence were harder to quantitate. The amounts of viral DNA persisting in Ad12-induced tumor cells were as high and also as variable from tumor to tumor as in different cell lines transformed by Ad12 in culture (Table I, column 9) (c.f. Stabel *et al.*, 1980).

The patterns of integration of Ad12 DNA in 39 different tumors

The patterns of integration of Ad12 DNA in 39 different tumors were analyzed by conventional blotting and DNA-DNA hybridization techniques (Stabel *et al.*, 1980). The tumor DNAs were cleaved with the EcoRI restriction endonuclease, and intact Ad12 DNA or cloned Ad12 DNA fragments were ^{32}P-labeled by nick translation and used as hybridization probes. The data obtained for 11 tumors were published elsewhere (Kuhlmann and Doerfler, 1982); the results from another 28 tumors are summarized schematically in Figure 2a and b. The Ad12-specific bands comigrating with the internal fragments of authentic Ad12 DNA (see maps in Figure 2) were proven to be these internal fragments. Off-size bands comprising viral DNA joined to cellular DNA and therefore not corresponding to any EcoRI fragments of virion DNA were identified using cloned terminal fragments of Ad12 DNA as hybridization probes (Figure 2).

A comparison of the hybridization data revealed that the distribution of the Ad12-specific off-size bands was different in all the tumors investigated. Thus, at least the macroenvironments at the sites of integration differed from tumor to tumor. Even in the tumors that contained a large number of Ad12 DNA copies per cell (Table I, column 9), relatively simple off-size band patterns prevailed, indicating that all copies

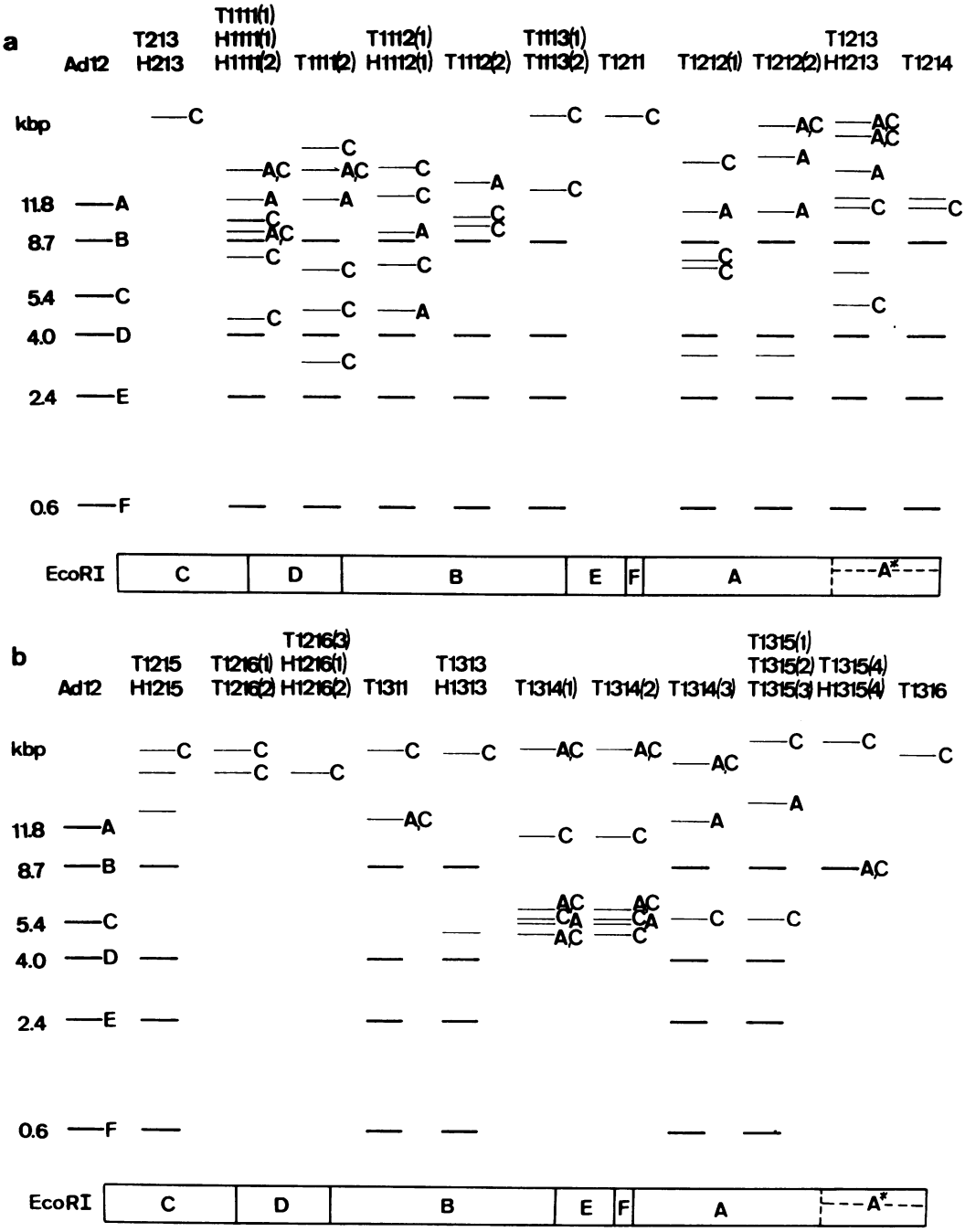


Fig. 2. Schematic presentation of the integration patterns of Ad12 DNA in the DNA of 26 different Ad12-induced hamster tumors and 10 hamster tumor lines. The patterns were determined by Southern blotting (1975) and DNA-DNA hybridization (Wahl *et al.*, 1979). As hybridization probes intact Ad12 DNA or the cloned terminal Ad12 DNA fragments A or C (see map) were used. All probes were ^{32}P-labeled by nick translation (Rigby *et al.*, 1977). Individual bands identified as homologous to these terminal fragments were designated A or C. In some instances, the same band hybridized to both the A and C fragments indicating a possible linkage between adjacent viral genomes. Bands not specifically designated comigrate with virion marker DNA bands and are identical with the corresponding marker DNA bands. Ad12 DNA was used as internal marker in (**a**) and (**b**). The sizes of the authentic Ad12 DNA fragments in kbp and the EcoRI map of Ad12 DNA are also shown.

of viral DNA were linked to cellular DNA at identical or at only a few different sites. Since the internal viral DNA fragments comigrated with the marker virion DNA fragments, we concluded that most of the viral DNA copies were integrated colinearly with virion DNA. In several tumors the off-size bands were relatively under-represented compared to internal viral DNA fragments. This suggests that either some of the off-size bands may be more randomly distributed or that internal parts of the Ad12 genome were selectively amplified. In many of the parameters mentioned here, the patterns of integration of viral DNA in tumor cells resembled those in transformed cells (Stabel *et al.*, 1980).

The apparently great variation in integration sites might be created by post-integrational events leading to the amplification of viral genomes and to rearrangements in the cellular genome. Perhaps, initially only one viral genome or a few copies of it were integrated at a very limited number of sites (Doerfler *et al.*, 1979; Neumann and Doerfler, 1981). The mechanisms of amplification and of re-arrangements of mammalian genes have not yet been elucidated and the adenovirus system may be well suited to study them.

In tumors T213, T1211, T1216, T1315(4), and T1316, only

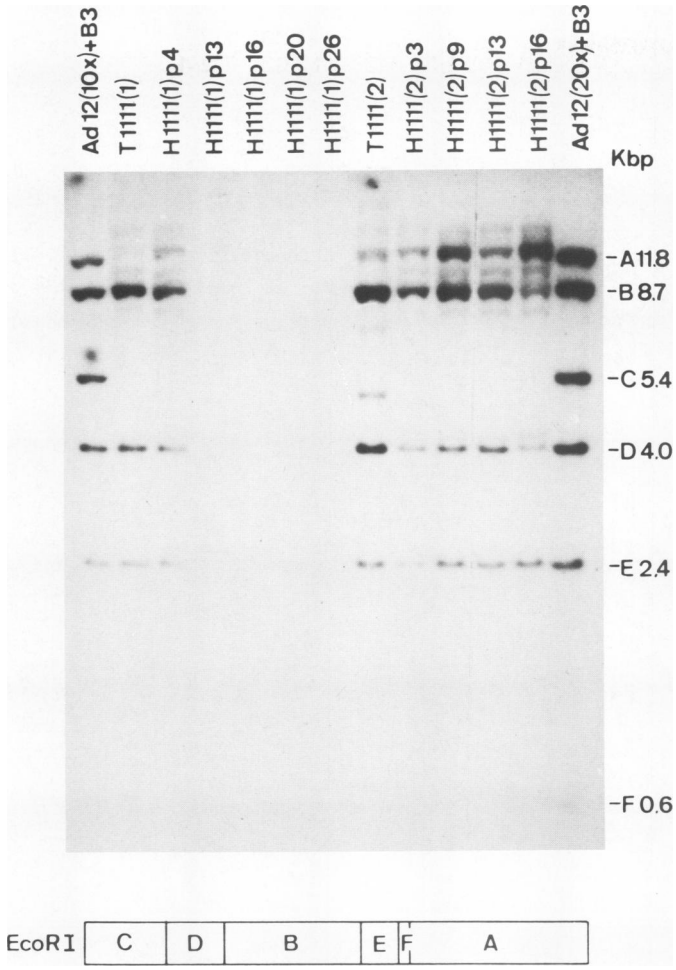


Fig. 3. Patterns of integration of Ad12 DNA in the DNA of Ad12-induced tumors and in tumor cell lines. The DNA of Ad12 (0.26 μg) mixed with 10 μg of B3 hamster DNA or 10 μg of DNA from tumors or cell lines as indicated was cleaved with the EcoRI restriction endonuclease. Fragments were separated by electrophoresis on 0.8% horizontal agarose slab gels, transferred to nitrocellulose filters, and hybridized to Ad12 DNA which was ^{32}P-labeled by nick translation. The designations T1111(1) and T1111(2) refer to two different tumors in one animal, H1111(1) and H1111(2) to cell lines established from these tumors, respectively, and carried between passage 4 (p 4) to 26 (p 26). All other details were as described in the legend to Figure 2.

off-size fragments of viral DNA homologous to the EcoRI C fragment could be detected. In tumors T1314(1) and T1314(2) derived from one animal, only multiple off-size bands were observed (Figure 2). Such patterns are unusual for Ad12 tumor cells and suggest that the viral DNA had been extensively fragmented. Such fragmentation is, however, frequently found in both rat and hamster cells transformed by Ad2 and Ad5 DNA (Sambrook *et al.*, 1974; Visser *et al.*, 1979; Dorsch-Häsler *et al.*, 1980; Vardimon and Doerfler, 1981).

Loss of the Ad12 genome and persistence of the tumor phenotype

In one hamster, two tumors, T1111(1) and T1111(2), were generated at the site of injection. The integration patterns of Ad12 DNA in the two tumors differed with respect to the number of off-size bands (Figure 3). From these tumors, respectively, cell lines H1111(1) and H1111(2) were established and passaged continuously. The persistence of the viral genome was examined at different passage levels. Cells of line H1111(1) in early passage (p 4) carried the Ad12 genome, whereas from passage 13 onward, it had been lost from these cells. Absence of Ad12 DNA was documented also by using the ^{32}P-labeled terminal fragments of Ad12 DNA as hybridization probes. The results of blotting experiments (Figure 3) were confirmed by measuring reassociation kinetics. Ad12 DNA could not be detected by this method either. Between passages 4 and 13, the Ad12 DNA must have been eliminated from the cellular genome. Concomitant with the loss of Ad12 DNA, cell morphology changed from epithelioid (Figure 4a) to fibroblastic (Figure 4c). In passage 12 the cell morphology changed abruptly (Figure 4b), and this change was paralleled in histological sections of the corresponding tumors (Figure 4d, e). From passage 12 of line H1111(1), 13 clonal sublines were established; five sublines were epithelioid, eight were fibroblastic. After another 6 weeks of continuous passage, all sublines were fibroblastic and viral DNA sequences had been lost from all of them. Cells of the H1111(1) line in passage 27 were injected into newborn hamsters. The fibroblastic revertants still proved oncogenic, although they had lost all traces of Ad12 DNA. The cells apparently retained a tumorigenic capacity in the absence of Ad12 DNA (Table II). Cell lines retaining Ad12 DNA were also tested for oncogenicity in newborn hamsters.

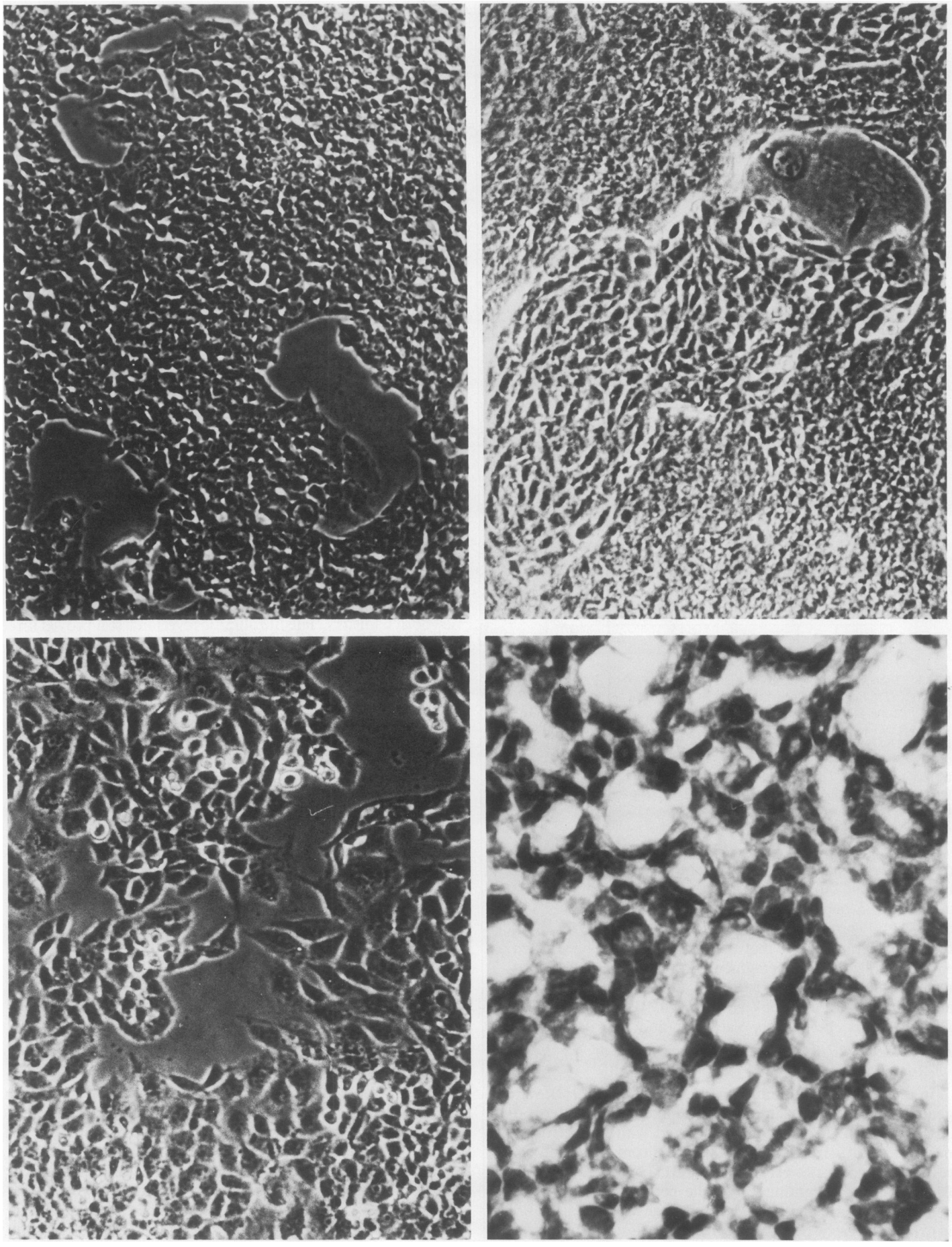
Table II. Oncogenicity in newborn hamsters of cell lines retaining and of cell lines devoid of Ad12 DNA

Cell line	Passage	Ad12 DNA[a] (copies/cell)	Number of cells injected	Tumor incidence	Time[b] (days)
H313	7	25	2 x 10^6	2/2	18
H1111(2)	14	10–11	2 x 10^6	8/8	18
H1111(1)	27	0	2 x 10^6	7/7	11

[a]The presence or absence of Ad12 DNA was ascertained by Southern blotting and DNA-DNA hybridization using ^{32}P-labeled Ad12 DNA as probe. The DNA of line H1111(1) was also tested by reassociation kinetics.
[b]Time period required for first appearance of tumors.

In a second tumor line H1111(2) established from tumor T1111(2) in the same animal that carried tumor T1111(1), the Ad12 genome equivalents were maintained (Figure 3), and the cells in passage 16 were still epithelioid. A comparison of the integration pattern of T1111(2) and of H1111(2) at passage 3 showed that two off-size bands with Ad12 DNA sequences had been lost in the established cell line. The factors affecting persistence or loss of viral DNA from Ad12-induced tumors are unknown but the use of conditioned or fresh culture medium might have a significant effect (Kuhlmann and Doerfler, 1982).

Cell line H313 was established from tumor T313 (Kuhlmann and Doerfler, 1982) and continuously passaged 30 times without reduction in the number of Ad12 genomes. From passages 7 and 10, a total of six clones was isolated and cells were grown to a high density. The cells from three clones were kept in fresh medium, the cells from the other three clones were maintained in conditioned medium from cultures of B3 hamster cells 24 h after passaging. After 6–7 weeks, the cells from the three clones kept in unconditioned medium had all reverted to fibroblastic morphology. One clone had lost all Ad12 DNA, a second clone retained 1–2 copies of Ad12 DNA out of 30 originally present, DNA from the third clone was not analyzed. The cells from the three clones propagated in conditioned medium did not change their morphology and kept all viral DNA sequences. These findings were consistent with the notion that loss of the viral genome led to a change in cell morphology. Fibroblastic but still on-



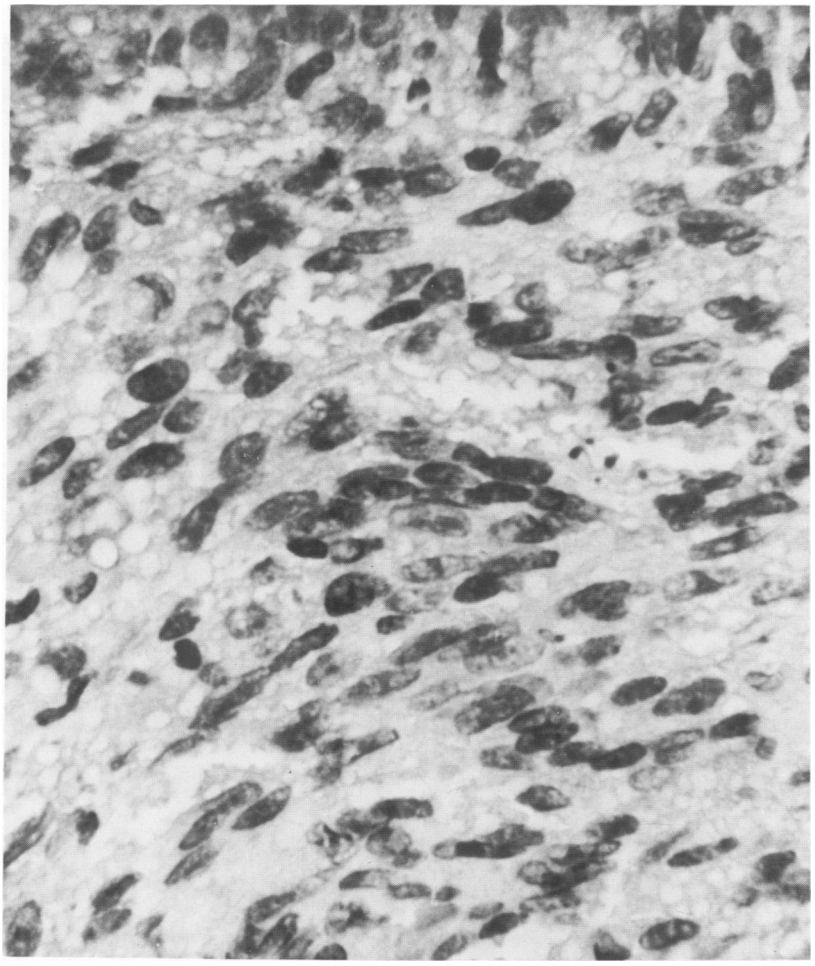


Fig. 4. Histologic preparations of tumors and photomicrographs of tumor cell lines in culture. **a, b, c,** Cell line H1111(1) in passages p 7, p 12, and p 16, respectively. Magnifications x 1600. Photographs of cell cultures were taken with a Leitz inverted microscope. **d,** Histologic section of T1111(1). Magnification x 2000. DNA from tumor T1111(1) contained viral DNA sequences (Figure 3). **e,** Cells from tumor T1111(1) were explanted into culture as cell line H1111(1) and carried up to passage 27. Ad12 DNA sequences were lost in passage 13 (Figure 3). Cells of p 27 were reinjected into a newborn hamster. Tumor T1716 was formed. Histological section of this tumor is shown. Magnification x 2000.

cogenic revertants of Ad12-transformed T637 cells, which had lost all or part of the Ad12 genome, have been described (Groneberg *et al.*, 1978; Eick *et al.*, 1980).

Discussion

The patterns of integration of Ad12 DNA in 39 Ad12-induced tumors (Figure 2; Kuhlmann and Doerfler, 1982) and in several Ad12-transformed and tumor lines (Stabel *et al.*, 1980), suggest that at least the macroenvironments at the sites of insertion into the cellular genome are all different. It is, however, still conceivable that the sites of primary integration of viral DNA may be more specific and that the apparent variety of insertion sites is the consequence of extensive amplification and reshuffling events. Most tumors, tumor lines, or transformed cell lines carry multiple copies of the intact Ad12 genome which are inserted in an arrangement colinear with that of virion DNA. It remains very difficult, particularly with tumor or transformed cell lines, to distinguish experimentally between random insertion of multiple copies of viral DNA, or specific insertion followed by amplification and rearrangements.

Although the injection of Ad12 into newborn hamsters induces tumors at the sites of injection in a large proportion (71%) of animals within a relatively short time (52 days) (Kuhlmann and Doerfler, 1982), it is surprising that metastases at remote sites have never been observed. Tumor cells can invade lymphatic vessels (Figure 1). We have observed nuchal tumors, when Ad12 stocks containing large amounts of SYREC particles (Deuring *et al.*, 1981) were used. It is possible that these nuchal tumors represent metastases in the regional nuchal lymph nodes. These findings raise the question to what extent human cellular DNA sequences in the SYREC DNA population can bestow properties on tumor cells that enable them to grow in local lymph nodes. The role of these cellular DNA sequences in adenovirus oncogenesis requires further investigation.

Perhaps the most remarkable finding is that the fibroblastic revertants derived from one tumor cell line, H1111(1), have retained the capacity to induce tumors in hamsters, although they have lost all Ad12 DNA sequences. The transient presence of Ad12 genomes in an integrated state in hamster cells may have fundamentally altered the cellular genome so as to cause and maintain cell oncogenicity. The Ad12 DNA appears to have behaved as a hit and run carcinogen. It is also worth emphasizing that the loss of the Ad12 genome is correlated with shifts from epithelioid to fibroblastic cell morphology.

In our experiments, cell morphology and the persistence of the integrated Ad12 genomes in explanted tumor cell lines

depended on the type of medium used. Fresh medium appeared to favor loss of viral genomes and fibroblastic morphology, whereas medium conditioned by hamster cells was correlated with persistence of viral DNA and epithelioid morphology. This interesting phenomenon requires further study.

Materials and methods

Ad12

The virus was propagated on human KB cells and was purified as previously described (Doerfler, 1969). Some of the Ad12 virus preparations contained detectable amounts of SYREC particles. SYREC DNA constitutes a symmetric recombinant between 750 to 1150 base pairs from the left terminus of Ad12 DNA and of cellular DNA (Deuring *et al.*, 1981). In some of the hybridization experiments, cloned fragments of Ad12 DNA were used (Vogel *et al.*, 1981).

Tumor induction in Syrian hamsters

Pregnant hamsters (*M. aureatus*, Han Aura) were obtained from the Zentralinstitut für Versuchstierkunde, Hannover, FRG. Newborn animals were injected with CsCl-purified Ad12 within 24 h after birth (Kuhlmann and Doerfler, 1982). Ad12 was injected s.c. in the lateral thoracic region in a dorsoventral direction.

Histology of tumor and organ tissues

Tissue specimens were fixed in 10% formaldehyde in phosphate-buffered saline (PBS), embedded in paraplast and stained with hematoxylin-eosin (Romeis, 1968).

Immune sera from tumor-bearing animals

The immune sera were obtained at the time when the hamsters were sacrificed. The sera were tested for the ability to precipitate Ad12-specific proteins from extracts of [^{35}S]methionine-labeled Ad12-transformed HA12/7 cells as described elsewhere (Achten and Doerfler, 1982).

Determination of the number of copies of Ad12 DNA in Ad12-induced tumor cells

This method was described earlier (Stabel *et al.*, 1980) and is outlined briefly in the legend to Table I.

Reassociation kinetics measurements

The technique of Gelb *et al.* (1971) was used, except that nick-translated ^{32}P-labeled viral DNA was used.

All other techniques

These have been outlined in detail earlier (Doerfler *et al.*, 1979; Stabel *et al.*, 1980).

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