Journal of Cellular Biochemistry 33:117–126 (1987)
Growth Factors, Tumor Promoters, and Cancer Genes 35–44

Mutations in the E1a Gene of Type 5 Adenovirus Result in Oncogenic Transformation of Fischer Rat Embryo Cells

Gregory J. Duigou, Lee E. Babiss, Wen-Shing Liaw, Stephen G. Zimmer, Harold S. Ginsberg, and Paul B. Fisher

Department of Microbiology (G.J.D., W-S.L., H.S.G., P.B.F.), and Department of Urology (G.J.D., P.B.F.), Comprehensive Cancer Center Columbia University, College of Physicians and Surgeons, New York, New York 10032, Rockefeller University, New York, New York 10021 (L.E.B.), Department of Medical Microbiology and Immunology, University of Kentucky Medical Center, Lexington, Kentucky 40536 (S.G.Z.)

Transformation of a specific clone of Fischer rat embryo (CREF) cells with wild-type 5 adenovirus (Ad5) or the E1a plus E1b transforming gene regions of Ad5 results in epithelioid transformants that grow efficiently in agar but that do not induce tumors when inoculated into nude mice or syngeneic Fischer rats. In contrast, CREF cells transformed by a host-range Ad5 mutant, H5hr1, which contains a single base-pair deletion of nucleotide 1055 in E1a resulting in a 28-kd protein (calculated) in place of the wild-type 51-kd acidic protein, display a cold-sensitive transformation phenotype and an incomplete fibroblastic morphology but surprisingly do induce tumors in nude mice and syngeneic rats. Tumors develop in both types of animals following injection of CREF cells transformed by other cold-sensitive Ad5 E1a mutants (H5dl101 and H5in106), which contain alterations in their 13S mRNA and consequently truncated 289AA proteins. CREF cells transformed with only the E1a gene (0–4.5 m.u.) from H5hr1 or H5dl101 also produce tumors in these animals. To directly determine the role of the 13S E1a encoded 289AA protein and the 12S E1a encoded 243AA protein in initiating an oncogenic phenotype in adenovirus-transformed CREF cells, we generated transformed cell lines following infection with the Ad2 mutant pm975, which synthesizes the 289AA E1a protein but not the 243AA protein, and the Ad5 mutant H5dl520 and the Ad2 mutant H2dl1500, which do not produce the 289AA E1a protein but synthesize the normal 243AA E1a protein. All three types of mutant adenovirus-transformed CREF cells induced tumors in nude mice and syngeneic rats. Tumor formation by these mutant adenovirus-transformed CREF cells was not associated with changes in the arrangement of integrated adenovirus DNA or in the expression of adenovirus early genes. These results indicate, therefore, that oncogenic transformation of CREF cells can occur in the presence of a wild-type

Received May 20, 1986; accepted July 10, 1986.

13S E1a protein or a wild-type 12S E1a protein when either protein is present alone, but does not occur when both wild-type E1a proteins are present.

Key words: integration of Ad5 DNA, primary transcription of Ad5 genes, CREF cells, cell transformation, Ad5 mutant genes, tumorigenicity, mutated Ad5 E1a gene

Although it is well established that viral and cellular oncogenes can independently or cooperatively induce cell transformation in vitro and that certain transformed cell populations can induce tumors when injected into susceptible animals, the underlying molecular mechanism(s) by which different transforming genes modulate the cellular phenotype have not been defined [1,2]. In the case of type 2 or type 5 adenovirus, complete morphological transformation of both primary and established cell cultures requires the expression of the early region 1a (E1a) and 1b (E1b) viral transcriptional units [3–11]. When a wild-type E1a gene is transferred into primary baby rat kidney cells it can immortalize these cells [12–14], but the cells acquire an incomplete transformed phenotype; ie, cells display a fibroblastic as opposed to an epithelioid morphology, and transformed cells are nontumorigenic [12]. In contrast, when primary baby rat kidney cells are cotransfected with a combination of E1a and the Ha-ras (T24) oncogene, complete oncogenic transformation results [13,14]. The ability of E1a to immortalize cells in culture and to cooperate with the Ha-ras oncogene in inducing oncogenic transformation is not unique to E1a since this function has been found with several other genes, including v-myc [15], c-myc [15], n-myc [16,17], polyoma large T-antigen [13,14], and the cellular tumor antigen p53 [18–20]. These studies have led to the concept that transformation of cultured primary cells requires at least two cooperating gene functions, ie, those responsible for immortalization and those responsible for transformation [14,15,21,22].

Recent studies have demonstrated that the biological phenotype induced by a specific oncogene depends not only on the level of its expression [23] or the expression of other cellular genes [24] but also on the target cell in which it is inserted [14,25,26]. For example, although a mutated or a normal Ha-ras gene transcriptionally activated by a long terminal repeat does not induce morphological transformation of primary rodent cells, these genes can immortalize primary cells [23]. Similarly, when c-myc or p53-cDNA genes are linked to strong viral promoters, they can induce oncogenic transformation of cells without dramatically altering cellular morphology [18,25,27]. In the case of adenovirus E1a, different effects have been observed depending on the target cell, including the induction of morphological transformation [7,9,11,28], cooperativity with Ha-ras in inducing transformation [13,14,29] (Duigou, Liaw, Babiss, and Fisher, unpublished data), and tumorigenic conversion [25]. In the established REF52 rat cell line, the combination of E1a and T24 Ha-ras genes was required to convert these cells to a tumorigenic phenotype [26], while in the 3T3 cell line a wild-type Ad5 E1a gene was sufficient to induce oncogenic conversion of these transformed cells [25]. In both REF52 and 3T3 cells insertion of E1a was not associated with dramatic alterations in cellular morphology, whereas in CREF [9] and the established 3Y1 [7] rat cell lines E1a induced morphologically transformed foci, but clones generated from these foci were not tumorigenic [26]. In contrast, when the Ad5 E1a 289 or 243AA protein is expressed alone in 3T3 cells [28] or when the levels of Ad2 E1a proteins are increased by substituting the strong promoter from the mouse metallothionein gene in place of the Ad2 E1a promoter [30], morphological changes are induced in NIH 3T3 cells. These studies demonstrate that the E1a gene

of Ad5 can do more than just immortalize cells, and they emphasize the importance of defining appropriate target cells and endpoints for analyzing the immortalization and/or transformation (morphological and/or oncogenic) potentials of specific genes or sets of genes.

In order to facilitate analysis of the mechanism by which adenovirus genes induce transformation, we have isolated and characterized a specific clone of Fischer rat embryo cells, called CREF [31,32], in which complete adenovirus virions [8–11,26,31–33] as well as transfected adenovirus transforming genes transform at high frequencies [9,11,34]. The CREF cell line has now been utilized for adenovirus transformation studies in several laboratories and has been shown to be valuable in defining the functions of specific areas of the E1a and E1b genes of adenovirus in regulating both the initiation and the maintenance of the transformed phenotype [10,11,33,35,36]. The CREF cell line, although immortal, exhibits many of the properties of normal rat embryo cells, including a high degree of contact inhibition, an inability to form macroscopic colonies when grown in agar suspension, and the inability to form tumors when inoculated into nude mice or syngeneic Fischer rats [26,32]. In the present study, we investigated the role of the 13S E1a encoded 289AA protein and the 12S E1a encoded 243AA protein in regulating the expression of the tumorigenic phenotype in transformed CREF cells.

MATERIALS AND METHODS

Cell Cultures

CREF cells [31,32] and the transformed CREF clones described in Table I were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum. The isolation and subsequent cloning of the cell lines used in these studies (Table I) have been described previously [8,9,26,32]. The Ad2 and Ad5 mutants and the plasmids containing various Ad5 transforming genes used in these studies have been described previously [8–11].

Tumor Induction in Nude Mice and Syngeneic Fischer Rats

The ability of cloned Ad5-transformed CREF cells to form tumors in 4-week-old Balb/c nude mice and 6–9-week-old immunocompetent Fischer 344 rats was determined by injecting 2×10^6 cells per animal. Monolayer cultures of tumor cells were established by trypsinization of excised tumors, and these are referred to as nmt (nude mouse tumor) or imm (immunocompetent rat tumor) cells.

DNA Analysis

High molecular weight DNA was isolated from the adenovirus-transformed CREF cell lines as previously described [37]. The presence of viral sequences within each cellular genome was determined by DNA filter hybridization analysis as previously described [31,38–40].

RNA Analysis

The in vitro nuclear run-off assay described by Hofer and Darnell [41] and Weber et al [42] was used to label nuclei from adenovirus-transformed CREF cells. The transcription assay utilized approximately 10^7 nuclei, which were incubated in the presence of 200 μCi of [^{32}P]-UTP (3,000 μCi/mmol) for 15 min at 30°C. Nuclear

TABLE I. Tumorigenic Properties of Wild-Type and Mutant Type 5 Adenovirus-Transformed CREF Cells

Cell line	Transforming agent[a]	Tumorigenicity[b]	
		Nude mice	Fischer rats
CREF[d]	None	0/9	0/6
wt3A[d]	H5wt	1/9 (90)[c]	0/6
c2[d]	H5wt 0–15.5 m.u. (E1a +E1b)	0/3	0/3
d2[d]	H5wt 0–15.5 m.u. (E1a + E1b)	0/6	0/6
hr1A2[d]	H5hr1	6/6 (18)	6/6 (42)
g8t[d]	H5hr1 0–15.5 m.u. (E1a + E1b)	9/9 (44)	0/6
S2	H5dl101 0–15.5 m.u. (E1a + E1b)	3/3 (24)	2/2 (91)
d3t[d]	H5hr1 0–4.5 m.u. (E1a)	6/6 (14)	3/3 (95)
01[d]	H5dl101 0–4.5 m.u. (E1a)	6/6 (27)	6/6 (157)
975-6	pm975 (No E1a 12S)	2/3 (78)	0/3
975-8	pm975 (No E1a 12S)	3/3 (30)	2/2 (113)
520-1	H5dl520 (No E1a 13S)	3/3 (34)	0/3
520-2	H5dl520 (No E1a 13S)	1/3 (39)	0/3
520-4	H5dl520 (No E1a 13S)	3/3 (44)	3/3 (105)
1500-2	H2dl1500 (No E1a 13S)	3/3 (76)	2/2 (87)
1500-6	H2dl1500 (No E1a 13S)	3/3 (25)	2/2 (128)
1500-10	H2dl1500 (No E1a 13S)	3/3 (11)	2/2 (71)
1500-11	H2dl1500 (No E1a 13S)	3/3 (19)	2/2 (108)

[a]H5wt: wild-type 5 adenovirus; H5wt 0–15.5: transfected CREF clone containing E1a and E1b (0–15.5 m.u.) of H5wt; H5hr1: host range mutant of Ad5 (contains a 1bp deletion of nucleotide 1055); H5hr1 0–15.5: transfected CREF clone containing E1a and E1b region of H5hr1; H5dl101 0–15.5: transfected CREF clone containing E1a and E1b region from H5dl101 virus (contains one 5bp deletion of nucleotides 1008–1012); H5hr1 0–4.5: transfected CREF clone containing E1a region of H5hr1; H5dl101 0–4.5: transfected CREF clone containing E1a region of H5dl101; pm975: host range mutant of Ad2 that lacks 12S E1a mRNA and the 243AA protein encoded by this mRNA; H5dl520: host range mutant of Ad5 that lacks a 13S E1a mRNA and the 289AA protein encoded by this mRNA; H2dl1500: host range mutant of Ad2 that lacks a 13S E1a mRNA and the 289AA protein encoded by this mRNA.

[b]Four-week-old Balb/C nude mice or 6–8-week-old Fischer 344 rats were injected with 2×10^6 cells. Results indicate No. of animals with tumors/ No. of animals injected, and bracketed No. indicates the average latency time in days, ie, first appearance of a palpable tumor. Nude mice were observed for tumor formation for $\geqslant 180$ days, and syngeneic rats were observed for tumor formation for $\geqslant 270$ days.

[c]This tumor developed late, was very small, and with further time it regressed.

[d]Tumorgenicity studies on these cell lines have been reprinted in [26].

RNA was isolated by the guanidinium isothiocyanate method of Ulrich et al [43], and prior to hybridization it was cleaved by treatment with 0.2 N NaOH for 15 min on ice [44]. The preparation of filters containing "dots" of denatured DNA was as described by Kafatos et al [45].

RESULTS

CREF cells transformed by complete Ad5 virions or the E1a and E1b genes from wild-type Ad5 failed to produce tumors when injected into either nude mice or syngeneic Fischer rats [26] (Table I). In contrast, CREF cells transformed by the host-range cold-sensitive mutant H5hr1 [8,46–49], or by similar mutants such as

H5dl101 and H5in106 [10], induced tumors in both nude mice and syngeneic rats [26] (Table I). These data suggest that the ability of these mutant-virus-transformed CREF cells to induce tumors in animals results from the defined mutation in the unique region of the E1a gene, which results in an altered 13S mRNA and consequently a truncated E1a 51-kd acidic polypeptide [47]. These results also indicate that tumorigenic transformation of CREF cells does not require E1b genetic information, since CREF cells transformed by only the E1a gene from H5hr1 or H5dl101 form tumors in both types of animals [26] (Table I).

To determine directly the role of the two E1a proteins, the 289AA (encoded by the 13S E1a mRNA) and the 243AA (encoded by the 12S E1a mRNA) protein, in regulating expression of the tumorigenic phenotype of CREF cells we have utilized the Ad2 mutant pm975, which produces only the 289AA E1a protein [10] and the Ad5 mutant H5dl520 [11] and the Ad2 mutant H2dl1500 [10], which produce only the 243AA E1a protein. Specific foci of CREF cells transformed by pm975 exhibited an epithelioid morphology similar to wild-type Ad5-transformed CREF cells but induced tumors in both nude mice and syngeneic Fischer rats (Table I). CREF cells transformed by H5dl520 and H2dl1500 displayed a cold-sensitive transformation phenotype, as did H5hr1-, H5dl101-, and H5in106-transformed CREF cells [8,9,48], and were tumorigenic in both nude mice and syngeneic rats (Table I). In the case of H5dl520-transformed CREF cells, cell cultures established from two different transformed foci, 520-1 and 520-2, induced tumors in nude mice (100% tumor incidence for 520-1 and 33% tumor incidence for 520-2) but did not form tumors in syngeneic rats. In contrast, the 520-4 transformed cell line produced tumors (100% of animals) in both types of animals. A different situation was observed in H2dl1500-transformed CREF cells in which all four transformed lines produced tumors in 100% of the nude mice and Fischer rats (Table I). Differences in latency time were observed in the formation of tumors in nude mice (11–44-day averages) and syngeneic rats (71–128-day averages) inoculated with the two types of mutant-transformed CREF cells. No direct correlation was observed between the latency period for tumor formation in nude mice and either the ability to form tumors or the latency time for tumor development in Fischer rats; eg, 520-1 formed tumors in three of three injected nude mice with an average latency period of 34 days but did not form tumors in rats, whereas 1500-2 formed tumors in three of three injected nude mice with an average latency period of 76 days and formed tumors in two of two injected rats with an average latency period of 87 days.

The process of tumor formation in both nude mice and syngeneic Fischer rats by the H5hr1-transformed CREF clone A2 was not associated with major alterations in the pattern of integration of viral DNA sequences, the number of integrated viral genomes, or the transcription rate of early viral gene regions, including E1a, E1b, E2a, E3, or E4 [26]. Similarly, the induction of tumors in nude mice and syngeneic rats by pm975-, H5dl520-, and H2dl1500-transformed cells also did not result in the rearrangement of integrated adenovirus DNA sequences (Fig. 1 and 2, and unpublished data). Subclones of H2dl1500-transformed CREF cells derived from the same original transformed focus; ie, 10-1 and 10-2, and 11-1, 11-2, 11-3, and 11-4, displayed similar patterns of DNA integration but differed in their tumorigenic potential (unpublished data).

To determine if tumor formation by H2dl1500 was associated with an alteration in the expression of viral genes other than E1a and E1b, the rate of transcription of

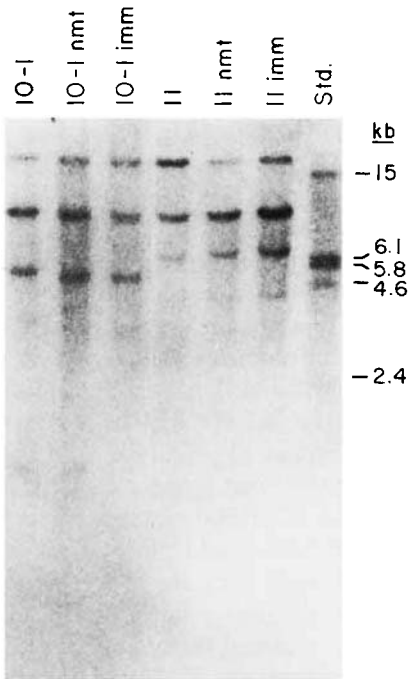

Fig. 1. Analysis of viral DNA in mutant adenovirus-transformed CREF cell lines. Cellular DNA (10 μg) was digested with XbaI, size-fractionated through 0.6% agarose, transferred to nitrocellulose filters, and probed using Ad2 DNA labeled with ^{32}P by nick-translation. 10-1 represents DNA of a cloned cell line isolated from an expanded focus of H2dl1500-transformed CREF cells. 11 contains DNA of a cell line derived from a focus of H2dl1500-transformed CREF cells. nmt and imm denote cell lines derived from tumors produced after injection of the different transformants into nude mice (nmt) or immunocompetent Fischer rats (imm). Std. contains 10^{-5} μg of XhoI-digested Ad5 DNA and is included as a size standard.

early viral genes was analyzed by labeling nascent RNAs from isolated nuclei with [^{32}P]-UTP and hybridizing to appropriate dots of DNA immobilized on nitrocellulose filters [26,41–45]. This procedure is useful for determining differential rates of RNA transcription initiation [41] since chain initiation in isolated nuclei is an inefficient process, whereas chain elongation of previously initiated RNA by RNA polymerase II occurs faithfully. By applying similar amounts of total labeled RNA (isolated from approximately equivalent numbers of cell nuclei) from each nuclear sample, the hybridization assays permitted a determination of the rate of transcription of the defined genes in each cell type. As previously found with the H5hr1-transformed A2 CREF clone, tumor formation by H2dl1500-transformed CREF cells followed by reestablishment in culture did not result in dramatic alterations in the rate of transcription of early adenovirus genes (Fig. 3). The two- to threefold decrease in the transcription rate of the E1a and E1b genes observed for the clone 11 and 11-imm cells compared to 11-nmt cells was not observed in another experiment. This conclusion is further supported by the increased hybridization signal for actin in the 11-nmt cell line.

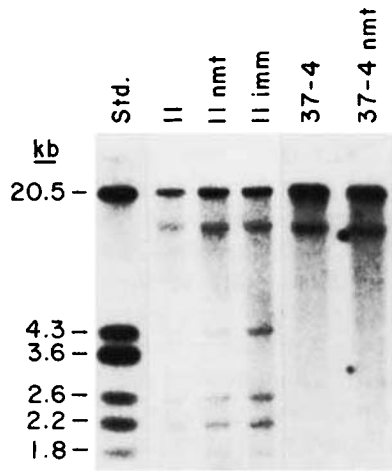

Fig. 2. Analysis of viral DNA in E1a 13S minus Ad2- and Ad5-transformed CREF cell lines. Cellular DNA (10 μg) was digested with EcoRI, size-fractionated through 0.6% agarose, transferred to nitrocellulose filters, and analyzed using ^{32}P-labeled Ad2 DNA. 11 contains DNA of a cell line derived from a focus of H2dl1500-transformed CREF cells. 37-4 represents DNA from a cell line expanded from a focus of H5dl520-transformed CREF cells. nmt and imm denote DNA from cell lines derived from tumors produced after injection of transformed CREF cells in nude mice (nmt) or immunocompetent Fischer rats (imm). Std. contains 10^{-5} μg of EcoRI-digested Ad2 DNA and is included as a size standard.

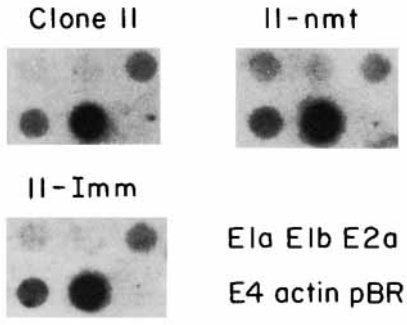

Fig. 3. Dot hybridization of in vitro labeled RNA isolated from cell lines clone 11, 11-nmt, and 11-imm. Nuclear RNA was labeled in vitro with ^{32}P-UTP as described in the text, and RNA containing 4×10^6 cpm was hybridized to a dot containing 7 μg of E1a (0–4.5 m.u.), E1b (7.9–9.6 m.u.), E2a (60.1–63 m.u.), E4 (93–100 m.u.), chicken actin cDNA, and pBR322 (pBR) DNA.

DISCUSSION

This study demonstrates that the 243AA protein encoded by the Ad5 E1a 12S mRNA, when present alone or in combination with a truncated form of the 289AA protein encoded by a mutated E1a-derived 13S mRNA, in concert with or without other Ad5 genes is capable of eliciting a tumorigenic phenotype in CREF cells. In addition, tumorigenic CREF cells were also generated following transformation by the Ad2 mutant virus pm975 [10], which expresses the E1a 289AA protein but not the E1a 243AA protein. Tumorigenicity was not associated with major changes in the integration profile of Ad5 DNA sequences or in the transcription rates of several

early viral and cellular genes. In contrast, when both wild-type E1a proteins, alone or in combination with E1b and other Ad5 gene products, are expressed in CREF cells, transformed clones do not produce tumors in either nude mice or syngeneic Fischer rats.

The mechanism is not known by which both wild-type E1a proteins repress the oncogenic phenotype in CREF cells, while alterations in the types of E1a protein(s) in CREF cells result in the tumorigenic conversion of these cells. Possible insights into this mechanism come from the observations that the E1a proteins exert pleiotropic responses in cells [14,28,47,50–53] and the 13S and 12S gene products, when present separately, may differ in their ability to elicit specific responses in target cells [52,53,57–62]. For example, it has been suggested that the 289AA E1a protein is more effective than the 243AA protein in transcriptionally activating other early Ad5 genes [47,50–53], such as E2, and nonviral genes [54–58], and that it is more active in inducing cell cycle progression [60]. In contrast, both the 13S and 12S gene products are similarly effective in immortalizing rodent cells [14], cooperating with the Ha-ras oncogene in inducing transformation in primary rodent cells [14], stimulating cellular DNA synthesis [10], and transcriptionally activating other adenovirus genes when microinjected as purified proteins into cells [61]. The diversity of E1a effects in cells is emphasized by the observations by means of DNA transfection techniques that E1a proteins can also inhibit transcription from the Ad2 E1a, SV4O, and polyomavirus enhancers [62,63], and the E2A late promoter [64]. The 13S and 12S gene products are similarly effective in suppressing transcription from the SV40 early promoter [63]. No studies have yet addressed the question of regulation of the expression of the 289AA and 243AA proteins when the genes coding for these proteins are expressed singly by transformed cells.

Recent studies by Schrier et al [65] and Bernards et al [66] demonstrate that 1) Ad12-transformed baby rat kidney cells lack at least two cellular proteins that are expressed in nononcogenic Ad5-transformed cells and in untransformed cells, and one of these proteins is encoded by the rat class I major histocompatibility complex; and 2) the product of the 13S mRNA of Ad12 may allow cells to escape T-cell immunity and may therefore be the mechanism by which these transformed cells induce tumors in syngeneic animals. Similarly, the importance of natural killer cells and macrophages in determining the tumorigenic potential of various DNA-virus transformed hamster and rat cells has been emphasized [67–72]. Based on these studies, a possible mechanism by which various CREF transformants that contain mutated E1a genes induce tumors in animals may involve the suppression of specific cellular surface antigens that mediate tumor cell rejection. Since transformed cells expressing both E1a proteins fail to induce tumors, the present results suggest that the combination of wild-type Ad5 E1a proteins may be unable to suppress the cellular genes effecting tumor rejection, whereas expression or overexpression [30] of either the 289AA or the 243AA protein alone may result in changes in cellular gene expression resulting in cell surface alterations mediating tumor formation. Support for this hypothesis comes from studies indicating that CREF cells transformed by the Ad2 mutant pm975 [10], which produces the 13S E1a-encoded 289AA protein and not the 12S E1a-encoded 243AA protein, as well as CREF cells transformed by the Ad2 mutant H2dl1500 [10] or the Ad5 mutant H5dl520 [11], which produce the 12S E1a-encoded 243AA protein and not the 13S E1a-encoded 289AA protein, are capable of inducing tumors in both nude mice and syngeneic rats (Table I). The present

challenge, therefore, is to determine the nature of the cellular alterations induced by various mutations in the E1a gene of Ad5 and Ad2 that result in expression of the tumorigenic phenotype by CREF cells.

ACKNOWLEDGMENTS

This work was supported by National Cancer Institute grants CA-35675 (P.B.F.), CA-33434 (S.G.Z.), and AI-12052 (H.S.G.). Dr. Lee E. Babiss was supported by a Damon Runyon-Walter Winchell Postdoctoral Fellowship (DRG-794). We thank A.J. Berk for providing the viral mutants pm975 and H2dl1500 and N.C. Jones for the Ad5 mutant H5dl520. We also thank Ms. Ellen Reynolds for assistance in the preparation of this manuscript and Mrs. C. Bruser for her technical expertise in the transformed cell tumorigenicity studies.

REFERENCES

1. Bishop JM: Annu Rev Biochem 52:310, 1983.
2. Bishop JM: Cell 42:23, 1985.
3. Gallimore PH, Sharp PA, Sambrook J: J Mol Biol 89:49, 1974.
4. Graham FL, Abrahams PJ, Mulder C, Heijneker HL, Warnaar SO, deVries FAJ, Fiers W, van der Eb AJ: Cold Spring Harbor Symp Quant Biol 39:637, 1974.
5. van der Eb AJ, Mulder C, Graham FL, Houweling A: Gene 2:115, 1977.
6. Jones N, Shenk T: Cell 17:683, 1979.
7. Shiroki K, Maruyama K, Saito I, Fukui Y, Shimojo H: Virology 138:1048, 1981.
8. Babiss LE, Ginsberg HS, Fisher PB: Proc Natl Acad Sci USA 80:1352, 1983.
9. Babiss LE, Fisher PB, Ginsberg HS: J Virol 49:731, 1984.
10. Montell C, Courtois G, Eng C, Berk AJ: Cell 36:951, 1984.
11. Haley KP, Overhauser J, Babiss LE, Ginsberg HS, Jones NC: Proc Natl Acad Sci USA 81:5734, 1984.
12. Houweling A, van der Elsen PJ, van der Eb AJ: Virology 105:537, 1980.
13. Ruley HE: Nature 304:602, 1983.
14. Zerler B, Moran B, Maruyama K, Moomaw J, Grodzicker T, Ruley HE: Mol Cell Biol 6:887, 1986.
15. Land H, Parada L, Weinberg RA: Nature 304:596, 1983.
16. Schwab M, Varmus HE, Bishop JM: Nature 316:160, 1985.
17. Yancopoulos GD, Nisen PD, Tesfaye A, Kohl NE, Goldfarb MP, Alt FW: Proc Natl Acad Sci USA 82:5455, 1985.
18. Eliyahu D, Raz A, Gruss P, Givol D, Oren M: Nature 312:646, 1984.
19. Parada L, Land H, Weinberg RA, Wolf D, Rotter V: Nature 312:649, 1984.
20. Jenkins JR, Rudge K, Currie G: Nature 312:651, 1984.
21. Land H, Parada LF, Weinberg RA: Science 222:771, 1983.
22. Newbold RF, Overell RW: Nature 304:648, 1983.
23. Spandidos DA, Wilkie NM: Nature 310:469, 1984.
24. Babiss LE, Guernsey DL, Fisher PB: Cancer Res 45:6017, 1985.
25. Kelekar A, Cole MD: Mol Cell Biol 6:7, 1986.
26. Babiss LE, Liaw WS, Zimmer SG, Godman GC, Ginsberg HS, Fisher PB: Proc Natl Acad Sci USA 83:2167, 1986.
27. Keath EJ, Caimi PG, Cole MD: Cell 39:339, 1984.
28. Roberts BE, Miller JS, Kimelman D, Cepko CL, Lemischka IR, Mulligan RC: J Virol 56:404, 1985.
29. Franza BR, Jr, Maruyama K, Garrels JI, Ruley HE: Cell 44:409, 1986.
30. Senear AW, Lewis JB: Mol Cell Biol 6:1253, 1986.
31. Fisher PB, Mufson RA, Weinstein IB, Little JB: Carcinogenesis 2:183, 1981.
32. Fisher PB, Babiss LE, Weinstein IB, Ginsberg HS: Proc Natl Acad Sci USA 79:3527, 1982.

33. Carter TH, Nicolas JC, Young CSH, Fisher PB: Virology 117:519, 1982.
34. Babiss LE, Fisher PB, Ginsberg HS: J Virol 52:389, 1984.
35. Winsberg G, Shenk T: EMBO J 3:1907, 1984.
36. Hurwitz DR, Chinnadurai G: Proc Natl Acad Sci USA 82:163, 1985.
37. Dorsch-Hasler K, Fisher PB, Weinstein IB, Ginsberg HS: J Virol 34:305, 1980.
38. Rigby PWJ, Dieckmann M, Rhodes C, Berg P: J Mol Biol 113:237, 1977.
39. Southern EM: J Mol Biol 98:503, 1975.
40. Wahl GM, Stern M, Stark G: Proc Natl Acad Sci USA 76:3683, 1979.
41. Hofer E, Darnell JE, Jr: Cell 23:585, 1981.
42. Weber J, Jelinek W, Darnell JE, Jr: Cell 10:611, 1977.
43. Ulrich A, Shine H, Chirgwin J, Picket R, Tischer E, Rutter WJ, Goodman HM: Science 196:1313, 1977.
44. Nevins JR: Methods Enzymol 65:768, 1980.
45. Kafatos FC, Jones CW, Efstratiadis A: Nucleic Acids Res 7:1541, 1979.
46. Graham FL, Harrison TJ, Williams JF: Virology 86:10, 1978.
47. Ricciardi RP, Jones RL, Cepko CL, Sharp PA, Roberts BE: Proc Natl Acad Sci USA 78:6121, 1981.
48. Ho YS, Galos R, Williams J: Virology 122:109, 1982.
49. Hermo H Jr, Babiss LE, Liaw WS, Pinto IM, McDonald RJ, Fisher PB: In Rein R (ed): "Molecular Basis of Cancer: Part A: Macromolecular Structure, Carcinogens and Oncogenes." New York: Alan R Liss, Inc., 1985, pp 489–512.
50. Berk AJ, Lee F, Harrison T, Williams J, Sharp PA: Cell 17:935, 1979.
51. Jones N, Shenk T: Proc Natl Acad Sci USA 76:3665, 1979.
52. Carlock LR, Jones NC: J Virol 40:657, 1981.
53. Montell C, Fisher EF, Caruthers MH, Berk AJ: Nature 295:380, 1982.
54. Treisman R, Green MR, Maniatis T: Proc Natl Acad Sci USA 80:7428, 1983.
55. Kao H-T, Nevins JR: Mol Cell Biol 3:2058, 1983.
56. Green MR, Treisman R, Maniatis T: Cell 35:137, 1983.
57. Svensson C, Akusjarvi G: EMBO J 3:789, 1984.
58. Stein R, Ziff EB: Mol Cell Biol 4:2792, 1984.
59. Gaynor RB, Hillman D, Berk AJ: Proc Natl Acad Sci USA 81:1193, 1984.
60. Bellett AJD, Li P, David ET, Mackey EJ, Braithwaite AW, Cutt JR: Mol Cell Biol 5:1933, 1985.
61. Ferguson B, Krippl B, Andrisani O, Jones N, Westphal H, Rosenberg M: Mol Cell Biol 5:2653, 1985.
62. Borrelli E, Hen R, Chambon P: Nature 312:608, 1984.
63. Velcih A, Ziff E: Cell 40:705, 1985.
64. Rossini M: Virology 131:49, 1983.
65. Schrier PI, Bernards R, Vaessen RTMJ, Houweling A, van der Eb AJ: Nature 305:771,1983.
66. Bernards R, Schrier PH, Houweling A, Bos JL, van der Eb AJ, Zijlstra M, Melief CJM: Nature 305:776, 1983.
67. Raska K, Jr, Dougherty J, Gallimore PH: Virology 117:530, 1982.
68. Raska K, Jr, Gallimore PH: Virology 123:8, 1982.
69. Cook JL, Hibbs JB, Lewis AM, Jr: Int J Cancer 30:795, 1982.
70. Sheil JM, Gallimore PH, Zimmer SG, Sopori ML: Immunology 132:1578, 1984.
71. Lewis AM, Jr, Cook JL: Science 227:15, 1985.
72. Sawada Y, Fohring B, Shenk TE, Raska K, Jr: Virology 147:413, 1985.