MOLECULAR AND CELLULAR BIOLOGY, Dec. 1983, p. 2298–2301
0270-7306/83/122298-04$02.00/0

Vol. 3, No. 12

NOTE

Presence of a Kirsten Murine Sarcoma Virus *ras* Oncogene in Cells Transformed by 3-Methylcholanthrene

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Received 16 June 1983/Accepted 6 September 1983

Oncogenes have previously been reported in the DNAs of mouse fibroblast lines which had become transformed after in vitro exposure to the carcinogen 3-methylcholanthrene. These oncogenes are now shown to be versions of the cellular Kirsten *ras* gene and are therefore homologous to oncogenes detected in a variety of human tumor DNAs.

An important advance in chemical carcinogenesis was the development by Sachs, Heidelberger, and their colleagues of in vitro systems for studying the action of carcinogenic compounds (3, 4, 17). These workers showed that cultures of rodent cells, such as mouse fibroblasts of the established C3H/10T½ cell line, respond in vitro to a variety of carcinogenic stimuli by producing transformed, tumorigenic colonies. This in vitro assay appears to recapitulate, at least in its outlines, the oncogenic conversion which occurs in the tissues of a carcinogen-treated test animal.

Because DNA appeared to represent an important target of carcinogens, experiments were undertaken to detect oncogenic information in the genomes of 3-methylcholanthrene (3-MC)-transformed mouse fibroblasts. The application of the DNAs of these cells to NIH 3T3 fibroblast cultures resulted in the outgrowth of transformants. This proved directly that the 3-MC-transformed cells carry oncogenic information in their DNA (20). These oncogenic sequences apparently arose in response to the exposure of C3H/10T½ cells to 3-MC, since DNAs of normal, untransformed C3H/10T½ cells induced no foci upon transfection. This indicated that activation of these oncogenes was induced by 3-MC, recognizing that this could result from a direct interaction between the carcinogen and a target proto-oncogene or from a complex cascade of events that was triggered initially by 3-MC.

The relationships between the 3-MC-induced oncogenes of three transformed C3H/10T½ cell lines and one BALB 3T3 cell line were subsequently explored by determining the presence or absence of restriction endonuclease sites in their respective oncogenes (22). These cleavage site determinations indicated that the four oncogenes were closely related to one another in structure. It was concluded that these four alleles arose independently from a common precursor sequence (22).

The nature of transfected oncogenes was further explored by a comparison with those oncogenes transduced by various acutely transforming retroviruses. These viral oncogenes are of cellular origin and were acquired by these viruses during their passage through various vertebrate hosts (5). Comparisons of transfected cellular oncogenes and retrovirus-associated oncogenes revealed that the same cellular proto-oncogene which became activated in humans as a bladder carcinoma oncogene was also activated in rats after its incorporation into Harvey murine sarcoma virus (6, 14, 18, 26). Other work showed a similar relationship between the oncogene of a lung, a colon carcinoma, an adrenocortical tumor, and that of Kirsten murine sarcoma virus (Ki-*ras*) (6, 11, 19).

These relationships were revealed by probing the DNAs of transfected cells with radiolabeled clones of various retrovirus oncogenes. An analysis of the DNAs of cells transformed by the oncogenes of the 3-MC tumor cell lines now gives an indication of a similar relationship. Two initial observations suggested to us that the 3-MC-activated oncogene was also a cellular Ki-*ras* (c-Ki-*ras*) gene. First, an analysis of the DNAs of cells transfected by the oncogenes of the 3-MC tumor lines shows increased copies of DNA segments reactive with the oncogene probe from Kirsten sarcoma virus (Fig. 1A).

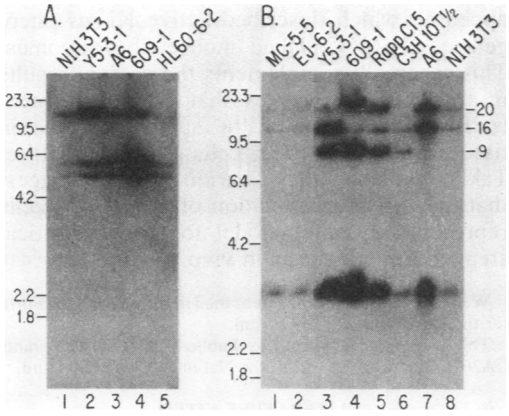

FIG. 1. Hybridization of a Ki-*ras* probe to DNA of transfected cells carrying oncogenes from 3-MC-transformed lines. A viral Ki-*ras*-specific clone, termed HiHi3 (7), was radiolabeled by nick translation and used as a probe to analyze a series of cellular DNAs. Ten micrograms of each cellular DNA was digested by the indicated endonuclease, subjected to gel electrophoresis, and transferred to nitrocellulose as described by Southern (24). (A) *Bgl*II digests of DNAs of the following cell lines: lane 1, untransfected NIH 3T3 cells; lane 2, Y5-3-1, a secondary transfectant derived from the transfer of the DNA of 3-MC-transformed C3H/10T½ cell line MCA Cl 16/39 (21, 22); lane 3, A6, a secondary transfectant derived from the transfer of the DNA of 3-MC-transformed BALB 3T3 line MC5-5 (2); lane 4, 609-1, a secondary transfectant derived from the transfer of the DNA of 3-MC-transformed C3H/10T½ MB66 MCA 609 (21, 22); lane 5, HL60-6-2, a secondary transfectant derived from the transfer of the DNA of human promyelocytic leukemia cell line HL60 (12). Size markers are *Hin*dIII-digested fragments of lambda phage DNA. (B) *Kpn*I digests of DNAs of the following cell lines: lane 1, MC5-5 (2); lane 2, EJ-6-2, a secondary transfectant derived from the transfer of the DNA of the EJ human bladder carcinoma (21); lane 3, Y5-3-1; lane 4, 609-1; lane 5, Rapp C15, a secondary transfectant derived from the transfer of the DNA of 3-MC-transformed C3H/10T½ line MCA5 (20); lane 6, untransfected C3H/10T½; lane 7, A6; lane 8, NIH 3T3.

Like other transfected oncogenes, these are amplified to multiple copies within the recipient cells, although they are present in single copy in the donors (13). Second, these additional c-Ki-*ras* sequences appear as high-molecular-weight fragments (>23 kilobases) after cleavage by *Bam*HI, *Sal*I, or *Xho*I endonuclease (data not shown). This is reminiscent of the behavior of the oncogenic sequences in these 3-MC-transformed cell lines, the biological activity of which was unaffected by treatment with any of these enzymes before transfection (22).

We wished to prove that these extra c-Ki-*ras* segments represented acquired copies of donor cell oncogenes and not simply extra copies of recipient cell sequences. Resolution of the transfected oncogene from the resident homolog of the recipient genome was difficult because both the donor cells and the NIH 3T3 recipients were of mouse origin. A distinction between donor and recipient genes was made possible by the finding of a restriction site polymorphism in the c-Ki-*ras* genes. There is at least one more *Kpn*I endonuclease site in the C3H/10T½ donor allele than in the corresponding allele of the NIH 3T3 recipient. Thus, as shown in Fig. 1B, *Kpn*I leaves a 20-kilobase c-Ki-*ras* segment in NIH 3T3 DNA (lanes 2 and 8) which corresponds to a 9-kilobase segment in the DNA of C3H/10T½ cells (lane 6).

An examination of the DNAs of 10 independent transfectants, examples of which are shown in Fig. 1B, lanes 3 to 5, shows that the transfectants have all acquired a novel DNA segment which comigrates with the 9-kilobase band of the C3H/10T½ donor tumor cells. We conclude that the additional c-Ki-*ras* sequences are of donor tumor cell origin. Thus, the oncogenes detected by transfection in the DNAs of the 3-MC-transformed cell lines (22) are all allelic versions of cellular Ki-*ras* proto-oncogenes. As a further control, we analyzed the DNA of an NIH 3T3 transfectant carrying an oncogene from a 3-MC BALB 3T3 tumor cell line. The BALB 3T3 line (MC5-5; lane 1) and NIH 3T3 (lane 8) oncogenes exhibit no *Kpn*I cleavage site polymorphism, and as expected, the derived transfectant (lane 7) shows fragments identical in size to those of both the donor and the NIH 3T3 recipient.

We note that the oncogenes are not amplified (e.g., Fig. 1B, lane 1) within the donor cells any more than in the control NIH 3T3 cells (Fig. 1B, lane 8). This indicates that the activity of the oncogene within the donors is likely not attributable to any amplification in copy number (11, 19) but rather must be due to an alteration in the single-copy gene. Such an alteration might be one that affects the amino acid sequence of the encoded protein, as has been found in the oncogene of the T24/EJ human bladder carcinoma (16, 25, 26).

We also observed increased levels of Ki-*ras* homologous RNA (Fig. 2A) and p21 protein (Fig. 2B) in transfected cells compared with their untransfected counterparts. By analogy with previous work (25), we conclude that these p21 proteins are encoded by the oncogene and are likely to mediate portions of the transformed phenotype in both donor and recipient cells.

The identification of the transfected oncogenes with a Ki-*ras* sequence extends previously reported work (1) which showed that another *ras* proto-oncogene, namely, a cellular Harvey *ras* gene, can become activated both by retroviruses

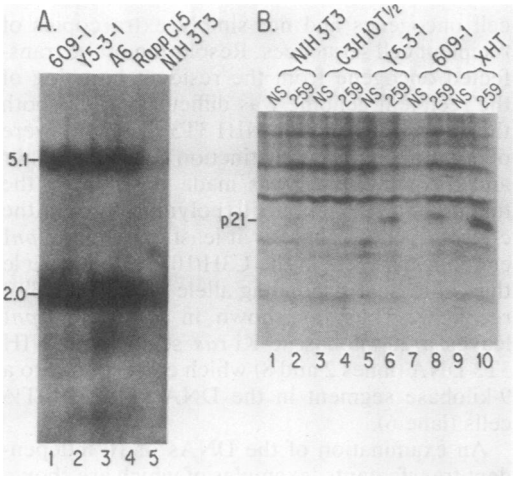

FIG. 2. Analysis of RNAs and proteins of transfected cell lines. (A) Cellular polyadenylated RNAs analyzed using the viral Ki-*ras* probe HiHi3 (7). The polyadenylated RNAs were prepared as described previously (14). See the legend to Fig. 1 for a description of cell lines from which RNA was prepared. These cell lines were: lane 1, 609-1; lane 2, Y5-3-1; lane 3, A6; lane 4, RAPP C15; lane 5, NIH 3T3. Molecular weights were determined by comparison with markers obtained from in vitro runoff transcription of the adenovirus late promoter (10). (B) Immunoprecipitation of cell lysates with monoclonal antiserum Y13-259 (9). Cultures were labeled with [^{35}S]methionine; lysates were prepared and immunoprecipitated as described previously (25). The lysates of lanes 1, 3, 5, 7, and 9, labeled NS, were immunoprecipitated with nonimmune serum. The lysates of lanes 2, 4, 6, 8, and 10, labeled 259, were immunoprecipitated with Y13-259 serum. Lysates were prepared from the following cell lines: lanes 1 and 2, NIH 3T3; lanes 3 and 4, C3H/10T½; lanes 5 and 6, Y5-3-1; lanes 7 and 8, A6; lanes 9 and 10, XHT, derived by transfection of the Harvey sarcoma virus genome. The encoded protein of this virus is also recognized by the Y13-259 serum (9).

and by chemical carcinogens. The Ki-*ras* oncogene identified here appears to be the direct homolog of oncogenes previously found in tumors of the lung, colon, connective tissue, pancreas, and adrenal cortex (6, 8, 11, 15, 19, 23). Perhaps most importantly, these results show that in vitro chemical carcinogenesis leads to molecular alterations which are similar, if not identical, to those occurring in spontaneously arising human tumors. These Ki-*ras* oncogenes may have become activated by the direct action of 3-MC on the Ki-*ras* proto-oncogene DNA. Alternatively, the introduction of 3-MC into the mouse fibroblasts may trigger a complex series of events, one of which is the activation of this oncogene.

After the completion of this study, a report appeared which described active Ki-*ras* oncogenes in 3-MC-induced mouse fibrosarcomas. This report (8) complements the present results in that it demonstrates that 3-MC treatment leads to activation of the same oncogene in tissues or in C3H/10T½ cells grown in culture. Taken together, these various results suggest that in vitro transformation of C3H/10T½ cells represents a useful model for certain critical steps in tumorigenesis in vivo.

We thank R. Ellis for the gift of the HiHi clone and M. Furth for the gift of the Y13-259 serum.

This work was supported by Public Health Service grants CA26717 and CA14051 from the National Cancer Institute.

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