Pluripotent Teratocarcinoma – Simian Virus 40 Transformed Mouse Fibroblasts Somatic Cell Hybrids

Boccara, M., Kelly, F.

Départment de Biologie Moléculaire. Institut Pasteur. 25 rue du Dr. Roux. 75015 Paris. France

Introduction

Mouse pluripotent embryonal carcinoma cells have been shown to be resistant to infection with polyoma virus and simian virus 40 but become susceptible to both viruses when allowed to differentiate in vitro [1,15]. The mechanism for this restriction is unknown but the block in the infectious cycle has been located after penetration of the virus particles and before synthesis of the early T antigen [2,16].

In this paper we describe the properties of hybrids between embryonal carcinoma cells and SV40 transformed mouse fibroblasts. This cross was undertaken in the hope to obtain hybrids with an embryonal carcinoma phenotype and containing an integrated SV40 genome. Such hybrids would be useful to study the expression of viral genes in embryonal carcinoma cells and during their differentiation. It is known from previous studies that different types of hybrid cells can result from crosses between embryonal carcinoma cells and differentiated cells. Whereas fusion with thymocytes [10] or Friend erythroleukemia cells [11] can give rise to hybrids with an embryonal carcinoma phenotype, fusion with fibroblasts have so far only given fibroblastic hybrids [3,7,9]. It has been proposed that the hybrid phenotype might at least in part depend on the chromosome constitution of the differentiated parent: the subtetraploidy of the fibroblasts used in these studies could favour the expression of the differentiated function [10].

In the experiments reported here, we have chosen as the fibroblastic parent cells from the SVT2 line of SV40 transformed fibroblasts, because of their near diploid chromosome constitution [8]. We have not yet succeeded in obtaining a stable embryonal carcinoma like hybrid and most hybrids appear to be fibroblastic. However we show that it is possible to obtain a new class of hybrids which retain some of the properties of the embryonal carcinoma parent.

Results

The cell lines used are: 1. PCC4azal, an azaguanine resistant clone of the embryonal carcinoma line PCC4 [6] which is near diploid [4]; 2. A clone of the SV40 transformed fibroblast line SVT2 which is resistant to 30 μg/ml of 5-bromodeoxyuridine (BUdR).

Table 1. Chromosome constitution of hybrid and parent cells[a,b]

Chromosomes no. mean (range)	PCC4 markers[c] 1/1	13/13	SVT2 markers[c] 1/3	14/14	19/19	19/M1
PCC4 39 (39–40)	1	1	–	–	–	–
SVT2 40 (35–45)	–	–	1	1	1 or 19/M1	1 or 19/19
F10 68 (63–80)	1 or 0	1	1 or 0	1	1 or 19/M1	1 or 19/19
D2 74 (71–78)	1	1	1	1	0	1

[a] Cells were grown in Dulbecco modified Eagle's medium containing 10% foetal calf serum in an atmosphere of 12% CO_2 and at 37°C. 3.10^5 cells from both parents were seeded in the same 100 mm dish and fused according to the Polyethyleneglycol procedure (12). 24 hours later, they were seeded at lower density (2.10^5 cells per 100 mm dish). After an other day hybrid cells were selected in HAT medium (supplemented with 68 mg/l hypoxanthine. 0.88 mg/l aminopterin and 19 mg/l thymidine) with medium changes every two days. After two weeks the morphology of the colonies was examined and clones were isolated.

[b] Chromosome preparations were made according to a standard air drying procedure. Individual chromosomes and markers were identified by their G-banding pattern using the trypsin-Giemsa method (14).

[c] PCC4 markers (4) and SVT2 markers (8) have been described previously

Hybrids between cells from the two lines have been selected in HAT medium as described in legend for Table 1. Colonies arise at a frequency of about 1 in 10^4 cells, most of which resemble the fibroblastic parent. However one in ten colonies appears to be composed of cells with a different morphology. Colonies were isolated and two of them were chosen for further studies: F10 which is fibroblastic and D2 which is made of round and loosely attached cells.

It is shown in Table 1 that both clones are subtetraploid and contain marker chromosomes from both SVT2 and PCC4. They therefore appear to contain most chromosomes from both parents. This is particularly striking for the D2 hybrid which contains all marker chromosomes as well as four copies of most autosomes as illustrated in Fig. 1.

Some properties related to the transformed phenotype of the SVT2 cells are shown in Table 2. F10 is as tumorigenic as the SVT2 parent and the tumors produced are typical fibrosarcomas. The SV40 T antigen is expressed in 100% of the cells. Saturation density in 10% fetal calf serum does not correlate here with tumorigenicity and is ten times lower for the hybrid than for SVT2 cells. N2 is less tumorigenic and the tumors obtained are sarcomas of a different type. The T antigen is heterogeneous with only 80% of positive cells, approximately a third of which are weakly stained.

We have also examined the distribution of cell surface antigens which have been studied in the mouse teratocarcinoma system [5]: 1. The early embryonic F9 antigen which is common to all embryonal carcinoma cells tested, cleavage embryos and spermatozoa, but is absent from differentiated cells. 2. The H-2 antigen, from the major histocompatibility locus, which is present on a variety of differentiated cells and also appears on embryonal carcinoma cells when they are allowed to differentiate. Results in Table 2 show that the F10 hybrid carries the $H\text{-}2^d$ haplotype from SVT2 cells (Balb/c

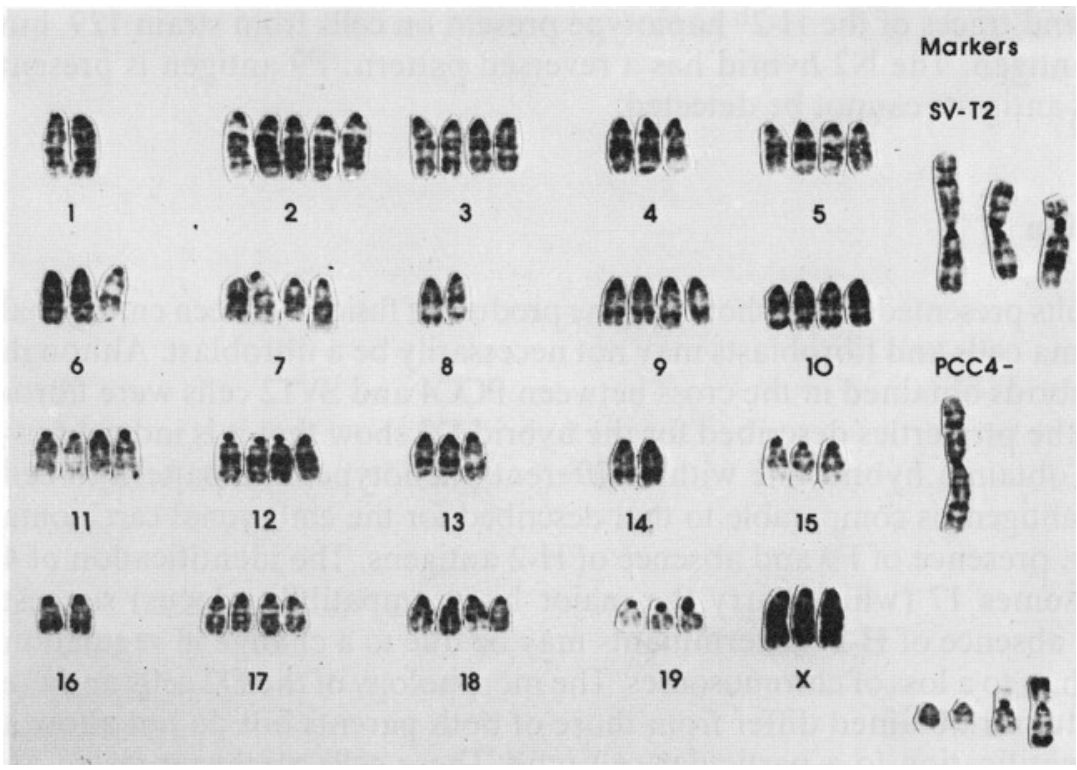

Fig. 1. Trypsin-Giemsa karyotype of one D2 hybrid cell

Table 2

Morphology		Tumorigenicity		SV40 T antigen	Saturation density	Cell surface antigens[d]		
		Efficiency[a]	Type of tumor[b]	% positive cells[c]	cells per $cm^2 \times 10^{-5}$	F9	H-2^d	H-2^b
PCC4azal	embryonal carinoma	high	embryonal carcinoma with multiple differentiations	0	ND	+	–	–
SVT2 BUdR^r	fibroblast	high	fibrosarcoma	100	10.6	–	+	–
F10	fibroblast	high	fibrosarcoma	100	1.1	–	+	traces
D2	round	low	polymorphic sarcoma	80	1.2	+	–	–

[a] Cells were injected subcutaneously and intraperitoneally in (129/SV × Balb/c) F1 hybrid mice irradiated with 600 rads from a cesium source. "High" refers to tumor formation in 100% of the mice after injection of 3.10^6 cells; "low" corresponds to tumor formation in 50% of the mice when injected with 10^7 cells.

[b] Histological preparations were made and examined by Dr. J. Gaillard (Institut Pasteur) as described previously (6).

[c] SV40 T antigen was detected by indirect immunofluorescence (1).

[d] Cell surface antigens were detected as described previously (5). In brief, the specific antisera were absorbed with the cells to be tested. Residual activity was examined in direct cytotoxicity tests with adsorbed rabbit complement on the appropriate cells: F9 cells for the anti-F9 serum, DBA/2 (H-2^d) and 129/Sv (H-2^{bc}) lymphocytes for the anti-H-2^d and anti-H-2^b sera respectively. Antisera were kindly given by P. Dubois (Institut Pasteur)

strain) and traces of the H-2^b haplotype present on cells from strain 129, but no F9 antigen. The N2 hybrid has a reversed pattern: F9 antigen is present but H-2 antigens cannot be detected.

Discussion

The results presented above show that the product of fusion between embryonal carcinoma cells and fibroblasts may not necessarily be a fibroblast. Although most hybrids obtained in the cross between PCC4 and SVT2 cells were fibroblastic, the properties described for the hybrid D2 show that it is indeed possible to obtain a hybrid line with a different phenotype. The pattern of cell surface antigens is comparable to that described for the embryonal carcinoma cells: i.e. presence of F9 and absence of H-2 antigens. The identification of 4 chromosomes 17 (which carry the major histocompatibility locus) suggest that the absence of H-2^d determinants may be due to a change in regulation rather than to a loss of chromosomes. The morphology of the D2 cells and the type of tumor obtained differ from those of both parents but do not allow a clear identification to a particular cell type. These cells might represent an intermediate state of differentiation. The use of a pseudo diploid fibroblast may have allowed the obtention of such cells.

The expression of the transformed phenotype of SVT2 cells is modified in the D2 hybrids as shown by decreased tumorigenicity and reduced expression of T antigen. The heterogeneous staining for T antigen resembles that described for SV40 intermediate transformed cells [13]. These results encourage us to think that such hybrids might be useful to study the effect of the host genome on the expression of viral genes. Further characterization of this and other independently isolated hybrids is in progress.

Acknowledgement

This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale (n° 76.4.311.AU and 76.4.483.C), the National Institutes of Health (CA 16355), the Fondation pour la Recherche Médicale Française and the Fondation André Meyer.

References

1. Boccara, M., Kelly, F.: Étude de la sensibilité au virus du polyome et à SV40 de plusieurs lignées cellulaires de tératocarcinome. Ann. Microbiol. (Inst. Pasteur) **129A**, 227–238 (1978)
2. Boccara, M., Kelly, F.: Expression of polyoma virus in heterokaryons between embryonal carcinoma cells and differentiated cells. Virology **90**, 147–150 (1978)
3. Finch, B.W., Ephrussi, B.: Retention of multiple developmental potentialities by cells of a mouse testicular teratocarcinoma during prolonged culture in vitro and their extinction upon hybridization with cells of permanent lines. Proc. Nat. Acad. Sci. USA **57**, 615–621 (1967)
4. Guénet, J.L., Jakob, H., Nicolas, J.F., Jacob, F.: Tératocarcinome de la souris: Étude cytogénétique de cellules à potentialités multiples. Ann. Microbiol. (Inst. Pasteur) **125A**, 135–151 (1974)

5. Jacob. F.: Mouse teratocarcinoma and embryonic antigens. Immunological Rev. **33**, 3–32 (1977)
6. Jakob. H.. Boon. T.. Gaillard. J.. Nicolas. J. F.. Jacob. F.: Tératocarcinome de la souris. Isolement. culture et propriétés de cellules à potentialités multiples. Ann. Microbiol. (Inst. Pasteur) **124B**, 269–282 (1973)
7. Jami. J.. Failly. C.. Ritz. E.: Lack of expression of differentiation in mouse teratoma-fibroblast somatic cell hybrids. Exp. Cell Res. **76**, 191–199 (1973)
8. Kelly. F.: Chromosome analysis of a simian virus 40 transformed mouse cell line and two variant sublines that are resistant to cytochalasin B. Cabcer Res. **35**, 1210–1213 (1975)
9. McBurney. M. W.: Clonal lines of teratocarcinoma cells in vitro. Differentiation and cytogenetic characteristics. J. Cell. Physiol. **89**, 441–455 (1976)
10. Miller. R. A.. Ruddle. F. H.: Pluripotent teratocarcinoma-thymus somatic cell hybrids. Cell **9**, 45–55 (1976)
11. Miller. R. A.. Ruddle. F. H.: Teratocarcinoma X Friend erythroleukemia cell hybrids resemble their pluripotent embryonal carcinoma parent. Develop. Biol. **56**, 157–173 (1977)
12. Pontecorvo. G.: Production of indefinitely multiplying mammalian somatic cell hybrids by polyethylene glycol (PEG) treatment. Somatic Cell Genet. **1**, 397–400 (1976)
13. Risser. R.. Pollack. R.: A non selective analysis of SV40 transformation of mouse 3T3 cells. Virology **59**, 477–489 (1974)
14. Seabright. M.: A rapid banding technique for human chromosomes. Lancet **1971 II**, 971–972
15. Swartzendruber. D. E.. Lehman. J. M.: Neoplastic differentiation: Interaction of Simian virus 40 and polyoma virus with murine teratocarcinoma cells in vitro. J. Cell. Physiol. **85**, 179–187 (1975)
16. Swartzendruber. D. E.. Friedrich. T. D.. Lehman. J. M.: Resistance of teratocarcinoma stem cells to infection with Simian virus 40: Early events. J. Cell. Physiol. **93**, 25–30 (1977)