Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

Tumors Induced in Hairless Mice by DNA from Human Malignant Cells

F. L. Kisseljov[1], L. Z. Topol[1], A. G. Tatosyan[1], N. G. Nalbandyan[1], E. S. Revasova[1] and M. Grofova[2]

The development of a transfection technique which is based on the transfer of foreign genetic material into permissive cells allows one to isolate and identify the transformed genes in tumors of different etiology [1]. The results which have been obtained in the last few years lead to the conclusion that primary effects in human tumors during their progression are connected with the activation of different genetic elements, among which oncogenes may play an important role [2]. The main aim of this work is the elaboration of an experimental model for the analysis of human tumors and human genome DNA fragments which participate in the process of malignant cell transformation.

The following human cell lines adapted for growth in vitro were analyzed: osteosarcoma U-393 OS; sinovial sarcoma U-4 SS; malignant glioma U-251 MG [3, 4]; fibrosarcoma B-6 FS [5], and bladder carcinoma T 24 [6]. Two primary human tumors were also included: stomach carcinoma; and testis carcinoma. DNA from tumors and cells was isolated by the SDS–phenol extraction method. The transfection of high molecular weight DNA was made according to well-known methods [7]. For the selection of transformed clones we used two methods: cultivation in vitro as described by Wigler et al. [8]; and a second method including the injection of the whole pool of transfected cells in hairless mice (2×10^6 cells per mouse) on the seventh day after transfection before any indication of morphological changes in cell phenotype.

As a result, we obtained around 30 DNA preparations from the foci of transformed cells and hairless mice tumors which were analyzed by the blot hybridization technique in the presence of *Alu* sequences, as a marker of human genome [9, 10]. Among these DNA preparations including T 24 DNA (which was used as a positive control), only ten were *Alu* positive and were used for further rounds of transfection. These *Alu*-positive DNA were again transfected in NIH 3T3 cells with further implantation in hairless mice. Results presented in Table 1 show that these DNA-induced tumors appeared in mice after a shorter period of time in comparison with the first round. The development of tumors having biologically inactive DNA in further cycles of transfection may be connected with the high background of spontaneous transformation observed in the clone of NIH 3T3 cells used for experiments.

The analysis of *Alu* sequences in DNA of murine tumors shows that after EcoR1 digestion, discrete bands of human genome were obtained. The pattern of these fragments, which arose after implantation of NIH 3T3 cells transfected with DNA from the malignant glioma cell line U-251 MG, was different after each round of transfection (Fig. 1a, lanes 1, 2, 3). So, after the second cycle, *Alu* sequences are associated with the bands of 3.2 megadaltons and after the third cycle 4.0 and 4.5 megadaltons. It indicates that, during DNA transfers from donor to recipient cells, structural re-

1 Cancer Research Center, Moscow, USSR
2 Cancer Research Institute, Bratislava

Table 1. Analysis of tumors in hairless mice, occurring after implantation of NIH 3T3 cells transfected with various human DNA

Number	DNA origin	Material	First round		Second round		Third round	
			Tumor appearance (days)	Alu sequences	Tumor appearance (days)	Alu sequences	Tumor appearance (days)	Alu sequences
1	Osteosarcoma	U-393 OS	35	+	–	–	–	–
2	Osteosarcoma	U-2 OS	35	+	–	–	–	–
3	Sinovial sarcoma	U-4 SS	33	+	22	+	20	+
4	Malignant glioma	U-251 MG	34	+	15	+	18	+
5	Fibrosarcoma	B-6 FS	20	+	12	N.T.[a]		
6	Bladder carcinoma	T 24	15	+	12	N.T.		
7	Stomach carcinoma	SC	35	+	–	–	–	–
8	Testis carcinoma	TC 87	72	–	–	–	–	–
9	Normal stomach tissue		–	–	–	–	–	–
10	NIH 3T3 cells		80	–	–	–	–	–

[a] N.T. not tested

arrangements of human genetic material may occur.

For tumors induced by U-4 SS DNA after the second round of transfection and EcoR1 DNA digestion, the main *Alu* band occurred at 4.5 megadaltons and diffuse low molecular weight bands were also visible (Fig. 1 a, lane 5). It is necessary to note that in tumors which developed after the first round, cell transfectants of the B-6 FS cell line *Alu*-specific sequences occurred as discrete bands at 13.7 and 4.5 megadaltons (Fig. 1 a, lane 8) although it is well known that, for the first round of transfectants, the diffuse distribution of *Alu* sequences is more typical [11].

Earlier it was shown that transformation of NIH 3T3 cells with DNA from tumors of different origin, including human bladder carcinoma, was accompanied by the transfer of the *ras* oncogene family and it was suggested that these genes may qualify as transforming genes of these tumors [12–14]. Therefore, the *ras* gene was chosen for analysis of the murine tumor genome. The results of these experiments are presented in Fig. 1 b. In all cases we observe hybridization with *ras*-specific probe and at the same time the main hybridizing fragments have the same mobilities as *Alu* fragments which testify to the human origin of these *ras*-specific sequences. Some additional bands which do not comigrate with *Alu* fragments where also observed. We suggest that they may represent either independent human *ras* sequences which are not linked with neighboring *Alu* sequences, or endogenous *ras* gene, for example, the 8.0 megadaltons fragment, which is also detected in NIH 3T3 cells (Fig. 1 b, lane 9). Densitometric scanning of autoradiograms of *Ha-ras* blots shows that, in every round of transfection, both the increase and decrease of integrated material may have occurred (it is well documented for the second and third rounds in U-251 MG cells).

In summary, we can conclude that the transfection of the DNA from human tumor cell lines U-251 MG, U-4 OS, and B-6 FS in NIH 3T3 cells with further implantation of these cells in hairless mice was accompanied by transfer of discrete regions of human genome which contains *ras* oncogene and highly repeated *Alu* sequences, both of which may undergo structural and qualitative changes, including amplification.

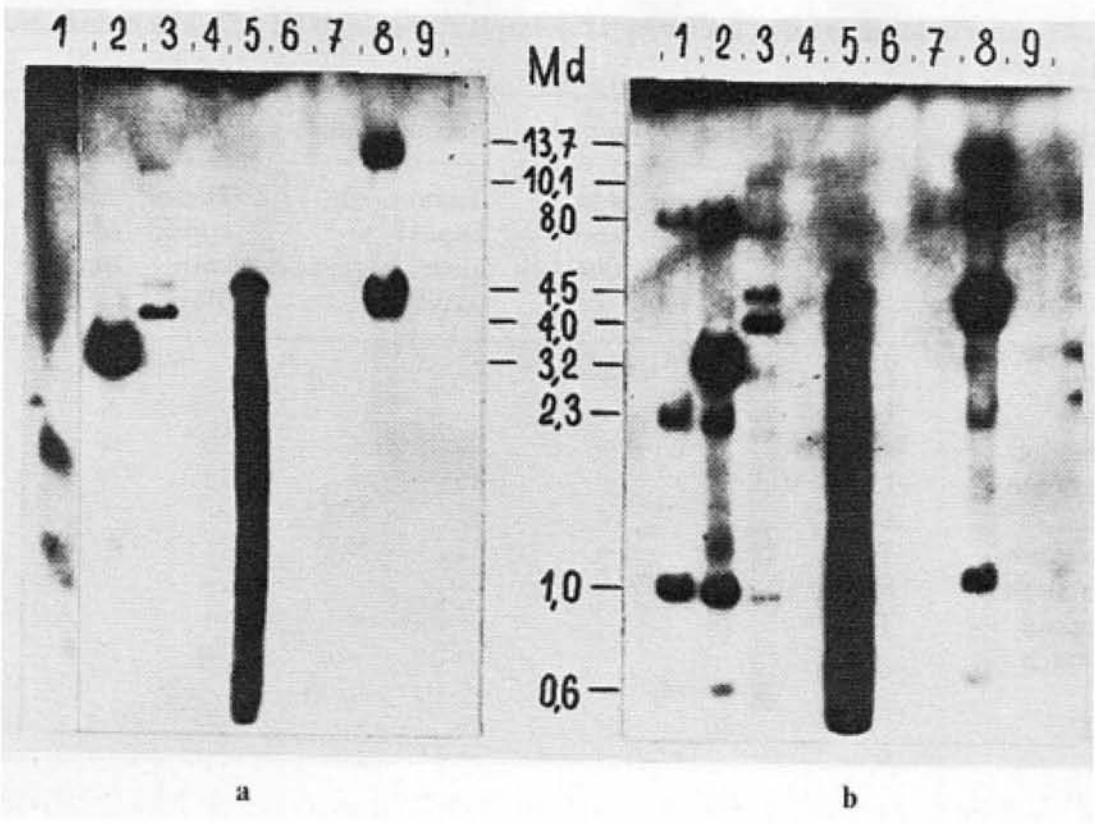

Fig. 1 a, b. Comparative analysis of hairless mice tumor genomes induced by implantation of NIH 3T3 cells transfected with various human DNA. **a** with *Alu*-specific probe, **b** with *Ha-ras*-specific probe. Human DNA for transfection was isolated from: 1, 2, 3 U-251 MG (malignant glioma) cell line (first, second, and third round of transfection, respectively), 4 testis carcinoma (first round); 5 U-4 SS (sinovial sarcoma) cell line (second round); 6 kidney of hairless mice; 7 tumor of hairless mice, induced by spontaneously transformed NIH 3T3 cells; 8 B-6 FS (fibrosarcoma) cell line (first round); 9 NIH 3T3 cells

References

1. Cooper G (1982) Science 217:801–806
2. Land H, Parada L, Weinberg R (1983) Science 222:771–778
3. Ponten J (1975) In: Human tumor cells in vitro. Plenum, New York, pp 175–178
4. Ponten J, Saksela E (1967) Int J Cancer 2:434–437
5. Thurso V, Popovic M, Matuska J, Grofova M (1976) Neoplasma 23:577–581
6. Bubenik J, Baresova M, Villicky V (1973) Int J Cancer 11:765–769
7. Graham FL, Van der Eb JA (1973) Virology 52:456–461
8. Wigler M, Sweet R, Sim GK (1979) Cell 16: 777–785
9. Southern EM (1975) J Mol Biol 98:503–507
10. Houck CM, Renehart FP, Schmid CW (1979) J Mol Biol 132:289–293
11. Peruchio M, Goldfarb M, Wigler M (1981) Cell 27:467–476
12. Shih C, Padhy LC, Murray M, Weinberg RA (1981) Nature 290:261–266
13. Krontiris TG, Cooper GM (1981) Proc Natl Acad Sci USA 78:1181–1184
14. Murray MJ, Shilo BZ, Shih C, Weinberg RA (1981) Cell 25:355–360