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Transforming Activity of DNA Extracted from Human Urological Tumours*

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Summary. The transforming activity of DNA extracted from one testicular cancer, one benign prostatic hyperplasia, two malignant prostatic adenocarcinomas, six renal cell carcinomas, two tumours of the renal pelvis, a specimen of normal urothelial tissue, two benign papillomas of the bladder and five malignant bladder carcinomas, was investigated using a DNA transfection assay on primary cultures of hamster kidney cells (HK cells) and mouse embryo fibroblasts (MEF cells). Transformation was scored under light microscope by the appearance of foci of morphologically transformed cells and by evaluating the ability of the transfected cells to from colonies in soft agar. Primary HK cells were unable to support transformation by human tumour DNA because of their very limited lifespan in vitro; MEF cultures, on the contrary, had a much more prolonged life: but were only abortively transformed by DNA from four out of six renal cell carcinomas and four out of seven malignant urothelial tumours, suggesting that transforming genes are present but not activated in the DNA from these urological malignancies.

Key words: DNA, Human urological tumours, Transfection, Transforming activity.

Introduction

Transforming genes from a number of human tumours including bladder, colon, lung and mammary carcinomas [6, 9, 12, 14, 20], B and T cell lymphomas [3, 10], sarcomas [11, 17] and others [2, 12, 14, 20] have been indentified in the last few years by several investigators using the DNA transfection assay on NIH-3T3 cells and blot-hybridization. It has been argued that transformation of NIH-3T3 cells upon transfection with human tumour DNA is due to the activation of transforming genes following the genomic rearrangement that occurs during DNA integration [5]; moreover, the analysis of NIH-3T3 cells transformed by human bladder carcinoma DNA revealed that the activated transforming gene was a cellular homolog of the ras^H gene of Harvey sarcoma virus [4, 13, 19].

We think that the demonstration of activated transforming genes in DNA of human urological tumours may be helpful in explaining the phenomenon of long-term carcinogenesis, frequently observed in the urinary tract. For this reason we tried to demonstrate the presence of transforming genes in human urological tumours by transfection assay on primary hamster and mouse cell cultures. We did not use NIH-3T3 cells for transfection experiments because we believed that these cells can easily detect the presence of transforming genes in human tumour DNA but, being a continuous cell line, are more prone than primary cells to undergo spontaneous transformation.

Material and Methods

DNA

The twenty surgical specimens used in this study for DNA extraction and transfection assay included: one mixed tumour of the testis (AC21), a benign hyperplasia of the prostate (MA22), two prostatic adenocarcinomas g.III (CG30, TL31-1), four renal cell carcinomas (BZ33, OA38, RE50, TE53), a relapsed renal cell carcinoma (CL43), one fibrosarcomatous renal cell carcinoma (CM36), a papillary carcinoma g.II of the renal pelvis (TL47), one squamous cell carcinoma of the renal pelvis (CV32), a specimen of normal urothelium (TL31-2), two benign papillomas of the bladder (AE29, TL40), two papillary carcinomas g.II of the bladder (VML45, FA51), one papillary carcinoma g.III of the bladder (BM44) and two squamous cell carcinomas of the bladder (MG28, BL37). The surgical specimens 0.5–1 cm thick were immediately frozen at −20 °C and kept frozen until used. DNA was extracted, purified, precipitated in $CaCl_2$ and transfected into primary cultures of hamster and mouse cells using techniques described in detail elsewhere [16].

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Table 1. Human tumour DNA transfection assay on primary MEF cultures

DNA extracted from			Transformation	
Patient	Organ	Histology and grading	Abortive	Stable
AC21	Testis	Mixed tumour: Embryonal carcinoma Seminoma Immature teratoma	No	No
MA22	Prostate	Benign hyperplasia	No	No
CG30		Adenocarcinoma g.III	No	No
TL31-1		Adenocarcinoma g.III	No	No
BZ33	Kidney	Renal cell carcinoma	Yes[a]	No
OA38		Renal cell carcinoma	No	No
RE50		Renal cell carcinoma	Yes[a]	No
TE53		Renal cell carcinoma	Yes	No
CL43		Renal cell carcinoma (relapse)	No	No
CM36		Renal cell carcinoma + fibrosarcoma	Yes	No
TL47	Renal pelvis	Papillary carcinoma g.II	Yes	No
CV32		Squamous cell carcinoma	No	No
TL31-2	Bladder	Normal urothelium	No	No
AE29		Benign papilloma	No	No
TL40		Benign papilloma	No	No
VML45		Papillary carcinoma g.II	No	No
FA51		Papillary carcinoma g.II	Yes	No
BM44		Papillary carcinoma g.III	Yes	No
MG28		Squamous cell carcinoma	No	No
BL37		Squamous cell carcinoma	Yes	No

[a] Morphological transformation

Cells

Hamster kidney cells (HK cells) were obtained by trypsinization of kidneys from 3–4 days old Syrian golden hamsters. Mouse embryo fibroblasts (MEF cells) were derived from skin, bones and muscles of 17–18 days old Balb/c mouse embryos. Cells were grown and maintained in Eagle's basal medium (BME) supplemented with 10% foetal bovine serum (FBS) (Flow Laboratories), 50 μg/ml gentamicin and 0.5 μg/ml amphotericin B.

Transformation Assay

Transformation was scored under light microscope by the appearance of foci of morphologically transformed cells and every weak by evaluating the ability of transfected cells to form colonies in soft agar (Difco) using a technique previously described [15].

Results

Primary HK cells resulted unable to support transformation by human tumour DNA because of their very limited lifespan in vitro.

MEF cultures had a much more prolonged life: DNA from human tumours, however, caused abortive transformation when transfected into these cells. Table 1 summarizes the results concerning transformation of MEF cultures. DNA extracted from testicular and prostatic tumours, either benign or malignant, did not transform normal cells in vitro; DNA from four out of six renal cell carcinomas caused abortive transformation, which was morphological transformation in two cases, BZ33 (Fig. 1A) and RE50 (Fig. 1B) as compared to control cultures (Fig. 1C). DNA from a papillary carcinoma of the renal pelvis caused abortive transformation when transfected into MEF cultures, while DNA from a squamous cell carcinoma of the same region caused no transformation. DNA from bladder urothelial tissue, either normal or hyperplastic, did not transform MEF cells, whereas DNA from two out of three papillary carcinomas of the bladder and only from one bladder squamous cell carcinoma could abortively transform normal mouse cells in vitro. In all these cases, abortive transformation was evaluated by the brief presence of colonies of transformed cells in soft agar (Fig. 2A, B, C).

Discussion

The inability of human tumour DNA or even human cloned transforming genes to cause stable transformation by transfection assay on cellular systems other than NIH-3T3 or analogous continuous cell lines has been recently documented [7, 8, 18]. Results reported here seem to confirm this

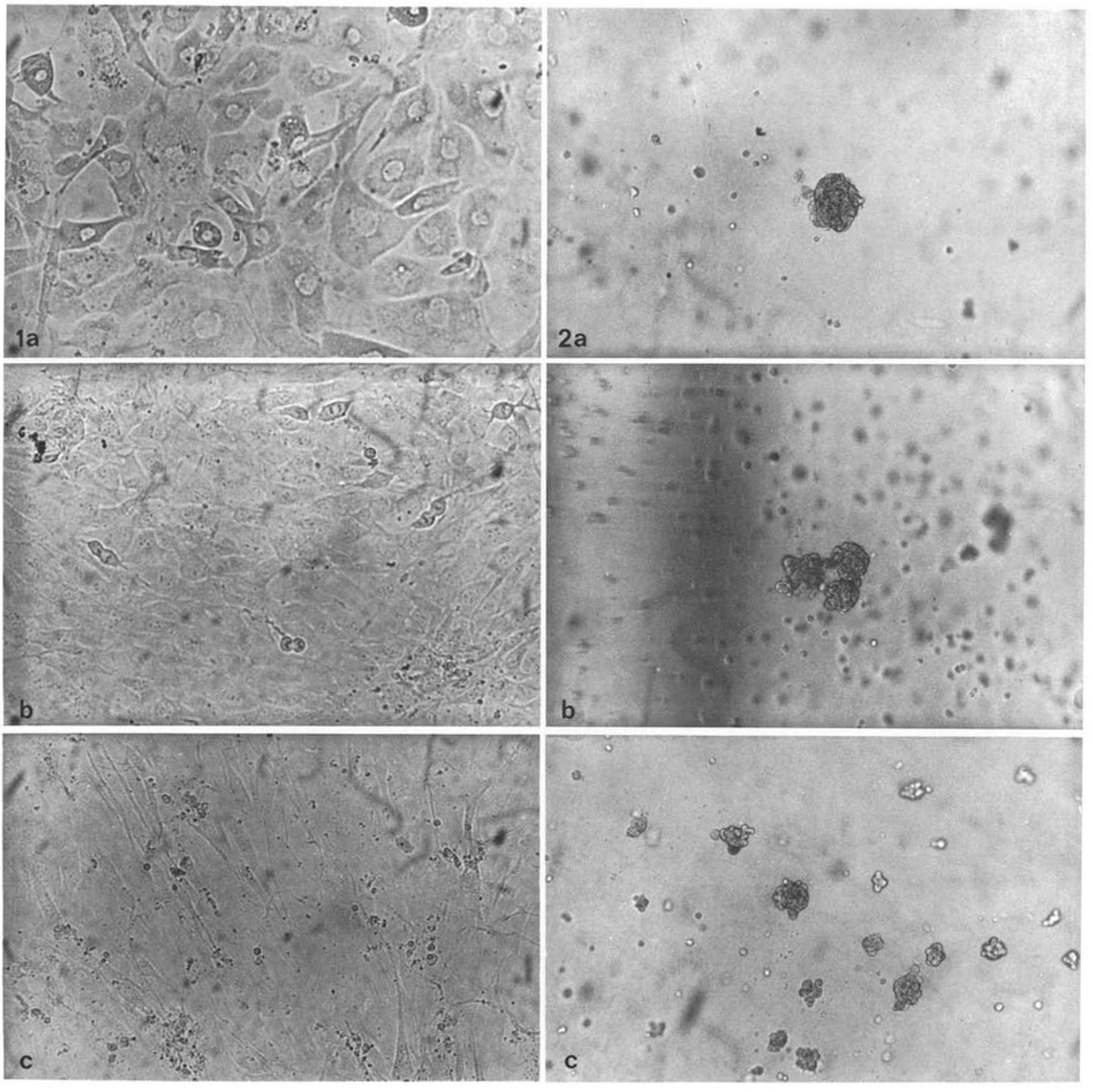

Fig. 1a–c. Light micrographs of MEF cultures transfected with DNA from BZ33 (a) showing large epithelial cells with clear nucleus and dark cytoplasm, and with DNA from RE50 (b) with small epithelial cells growing in colonies in a monolayer of normal fibroblasts, compared to control culture (c) showing a monolayer of normal fibroblasts (x200)

Fig. 2a–c. Colonies in soft agar formed by MEF cells transfected with DNA from TL47 (a) with one large colony, DNA from FA51 (b) showing three colonies of intermediate size, and DNA from BM44 (c) with numerous small colonies (x100)

phenomenon, which could be explained in several ways: one explanation is that NIH-3T3 cells, being a continuous cell line, have undergone mutations during their life in vitro which primed them for the acquisition of transforming genes, while primary normal cell cultures, not yet established or immortalized, cannot provide such cooperating alterations. Another possibility is that most human tumours contain oncogenes which can act as recessive or weakly transforming alleles and cannot be detected unless transfected into particular recipient cells. In our cellular system, we could obtain only abortive transformation upon transfection with human tumour DNA: such transformation was revealed by unstable acquisition by the transfected cells of the morphologically transformed phenotype and by the phenomenon of anchorage independence. We have not yet transplanted these cells into singeneic or nude mice in order to detect

the acquisition of the malignant phenotype, as reported by other authors [1].

Nevertheless, our results are of importance because we were able to obtain transformation, even if not stable, not only with DNA from human urothelial tumours, according to other investigators [6, 20], but also with human renal cell carcinoma DNA. Renal cell carcinomas are known to be hormone-dependent tumours and our future goal will be to investigate on the relationship between oncogene activation and hormone-dependency in these cancers.

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