Acta virol. 32 : 83—85, 1988

TRANSFORMING ACTIVITY OF CRUDE EXTRACT OF PSEUDORABIES VIRUS-TRANSFORMED CELLS

F. GOLAIS, *A. SABÓ, D. BAČÍKOVÁ

Department of Microbiology, Virology and Immunology, Faculty of Sciences, Comeniu s University, 817 03 Bratislava, and *Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

Received April 24, 1987

Summary. — Crude extract of pseudorabies virus (PRV)-transformed human (H-PR-1) cells induced transformation in human embryonic lung (HEL) cells. When the extract was removed, the acquired cell morphology remained unchanged, but the saturation density of cells was decreased. The transforming effect of the extract was neutralized with anti-PRV IgG.

Key words: pseudorabies virus-transformed cells; crude cell extract; transforming factor(s)

As reported previously, we were successful in transforming human cells with PRV. Prolonged cultivation of infected cells in the presence of antibody and human leukocyte interferon (IFN) resulted in appearance of transformed cells showing positive fluorescence of PRV antigen in nuclei and cytoplasma (Golais *et al.*, 1985). Next, we followed the replication of certain herpes viruses in these transformed (H-PR-1) cells as well as the effect of the soluble extract of H-PR-1 cells on HEL and other cells. The results from these experiments will be published elsewhere. In this paper we describe the effect of the crude extract of H-PR-1 cells on the transformation of HEL cells.

H-PR-1 cells were trypsinized and resuspended in serum-free culture medium to final concentration of $5 \times 10^7 - 1 \times 10^8$ cells/ml. The cell suspension was sonicated twice for 1 min and centrifuged at 100 000 × g for 60 min. The supernatant was dialysed against phosphate buffer saline for 72 hr, filtered through the 0.2 μm membrane filter (Sartorius) and stored at −70 °C until use.

Freshly trypsinized HEL cell suspensions (100 μl) containing 3×10^4 cells in culture medium were dispensed into wells of flat-bottom microplates. Undiluted cell extract (10 or 20 μl) was then added to each well. Part of the cells was infected with different viruses, other part which served as control remained non-infected (Golais *et al.*, manuscript in preparation).

In non-infected cells inoculated with the extract, morphological signs of transformation were observed after 7—10 days in culture. The cells

Table 1. The saturation densities of transformed HEL passaged in the presence and absence of extract

Passage	Extract present	Extract removed
1	2650*	1670*
2	2880	1050
3	3230	750
4	3380	510
5	3260	520

*Average values from 3 repeated experiments

became thin and elongated, some of them detached (Fig. 2). To exclude a possible toxic effect of the extract, same cells were treated with a crude extract of HEL and human melanoma (HMB-2) cells (Švec *et al.*, 1987); no morphological changes were observed.

After prolonged incubation in the presence of the extract (for 20—25 days), small foci of cells (2—5 foci in each well) were distinguished from other cells by altered morphology and "criss-cross" pattern of growth. All cultures containing foci were trypsinized, pooled and cultivated in the presence of the extract. The transformed phenotype of these cells remained preserved (Fig. 3).

In further experiments some properties of cells passaged in the presence and absence of the extract were compared. Removal of extract did not result in reappearance of original cell morphology (Fig. 1), only a gradual decrease in saturation density was observed and "criss-cross" pattern of growth was not as marked as in cells cultivated in the presence of extract (Fig. 4).

The saturation densities of cells (in cell number, per mm^2) after three days in culture are given in the Table, 1. No nuclear, cytoplasmic, or membrane-associated PRV antigen was demonstrated in these cells by direct immunofluorescence.

The extract had lost its transforming activity, when incubated for 90 min with 10% anti-PRV IgG possessing 1 : 5000 neutralizing antibody titre. Similarly, the transformed cells cultivated with the extract treated with IgG behaved like cells cultivated in an extract free medium.

The character and properties of the factor/s/ with transforming activity present in crude extract of H-PR-1 cells are unknown at present. It was shown that certain transformed cells produce growth factors which elicit cellular transformation when added to normal non-transformed cells. This special class of growth factors was termed the "transforming growth factors" (TGFs) (Lawrence, 1985; Massague, 1985). It was shown that TGFs are not virus coded but cell coded. However, the transforming effect of the factor/s/ present in the extract of H-PR-1 cells was neutralized with anti-PRV IgG indicating, that a PRV genome coded product might be involved in their formation in the transformed cells.

At present, experiments are carried out to scrutinize this interesting phenomenon.

References

Golais, F., Sabó, A., and Volná, A. (1985): Transformation of human embryonic cells by pseudorabies virus in the presence of antibody and interferon. *Biológia* (*Bratislava*) **40**, 1175—1181.

Lawrence, D. A. (1985): Transforming growth factors — an overview. *Biol. Cell* **53**, 93—98.

Massague, J. (1985): The transforming growth factors. *Trends Bioch. Sci.* **10**, 237—240.

Švec, J., Švec, P., Marchetti, A., and Squartini, F. (1987): Membrane-associated proteins of human melanoma cells: synthesis and processing influences by lipophylic substances. *Neoplasma* (in press.).

Explanation of Figures (Plate XII):

HEL cells cultivated in the presence or in the absence of the extract of H-PR-1 cells. × 1500

Fig. 1. Control HEL cells.

Fig. 2. Morphological changes in HEL cells after 10 days incubation with the extract.

Fig. 3.. Transformed phenotype appearing after prolonged incubation of HEL cells in the extract containing medium

Fig. 4. The same cells by 2nd passage after extract removal