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Retrovirus Particle Production in Three of Four Human Teratocarcinoma Cell Lines

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A. Introduction

In spite of a long and intensive search, human retroviruses have not yet been demonstrated unambiguously (for review see Pimentel 1979). The electron microscopic detection of retrovirus particles in human term placentas by Kalter and others (Kalter et al. 1973; Vernon et al. 1974) represents at present the most convincing piece of evidence that human oncornaviruses may exist. As our attempts to isolate these viruses from placentas or to cultivate placentas in vitro have not succeeded, tumors containing tissues histologically similar to that of the placenta were investigated for virus expression. Among other tumors, teratocarcinomas from males and choriocarcinomas from females were studied. In contrast to choriocarcinomas, most of the teratocarcinomas can be induced to produce retrovirus particles in tissue culture.

B. Methods and Results

Specimens of human testicular teratocarcinomas were taken immediately after extirpation, cut into small pieces, and incubated at 35° C in Dulbecco's modified Eagle's minimal essential medium supplemented with nonessential amino acids, 20% heat-inactivated fetal calf serum, 10% tryptose phosphate boullion, and antibiotics. Within a few weeks, fibroblasts and epithelial cells grew out as monolayers. Thereafter, the epithelial cells began to form domes and free-floating vesicles, which could be aspirated and subcultivated to obtain cell cultures free of fibroblasts. Two of the cell lines established in this manner at Tübingen are designated GH and HL. The two other human teratocarcinoma cell lines, Tera-1 and Tera-2, were kindly donated by J. Føgh (New York), who had established these lines from pulmonary metastases in 1970 and 1971, respectively (Føgh and Trempe 1975).

The initial search for retrovirus particle expression in Tera-1 and Tera-2 cells by electron microscopy revealed only a single particle with an equivocal morphology of retroviruses in a Tera-1 culture. This result parallels the essentially negative findings of Føgh and others (Bronson et al. 1979; Føgh and Trempe 1975). After treatment of the cultures with iododeoxyuridine (IUdR, 20 μg/ml), dexamethasone (DXM, 10^{-6}M), and dimethylsulfoxide (DMSO, 1%) for 24 h, retrovirus particles could be easily detected in three of the four teratocarcinoma cell lines investigated (Fig. 1 and Table 1). All other human cell cultures, including choriocarcinomas, seminomas, fibroblasts, melanomas etc., remained virus negative even after the induction regime.

The observed particles resemble C-type retroviruses but share a unique morphologic feature with viruses found budding from the syncytiotrophoblast of human term placentas (Dalton et al. 1974) in that their core appears adjacent to the virus envelope without the usual intermittent space seen with C-type virus particles. Similar morphologic observations have been made by Bronson using human teratocarcinoma cell cultures (Bronson et al. 1978, 1979). The missing electron lucent space between envelope and nucleocapsid may either be a property of the budding particles or may be the result of cellular influences on the budding process. We have never seen a single "mature" particle with a condensed core in all teratocarcinoma cell cultures investigated.

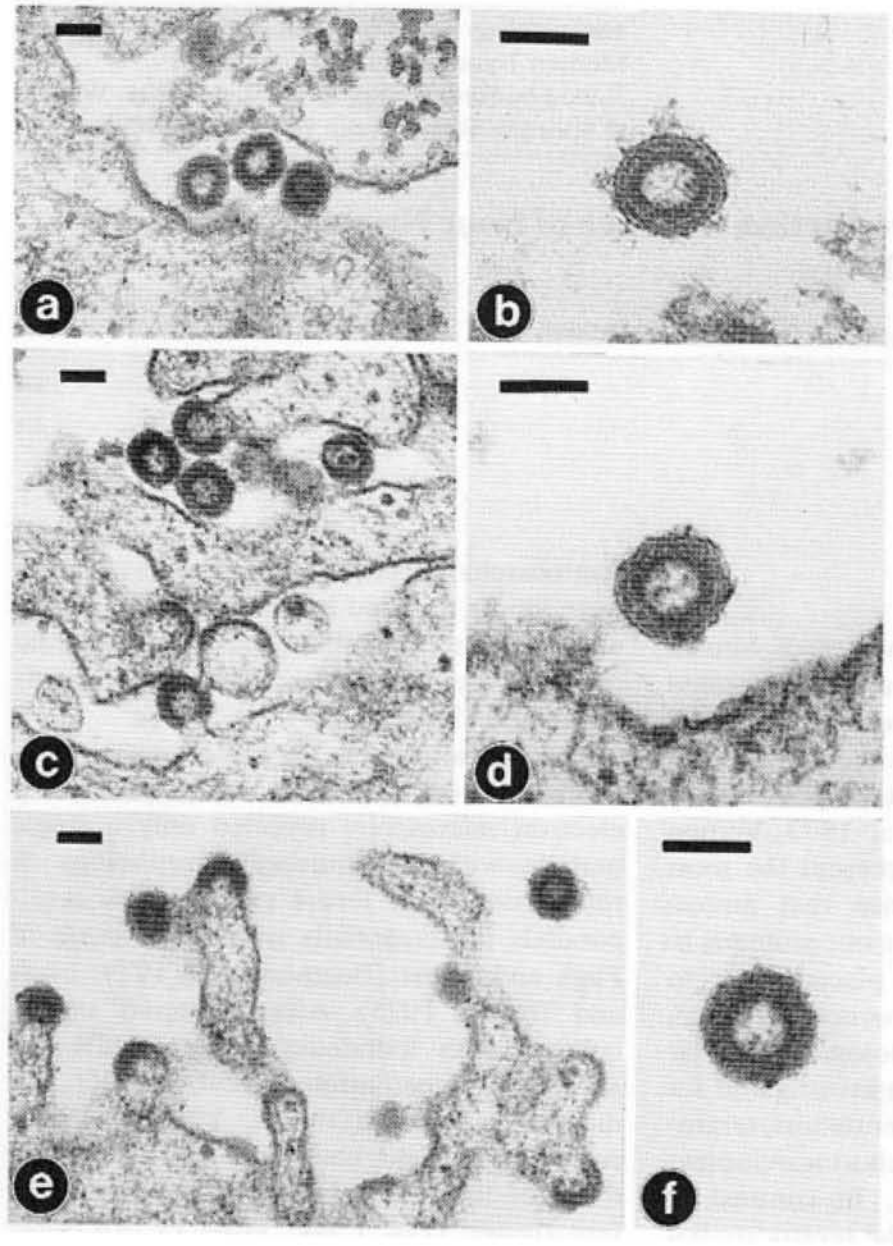

Fig. 1a–f. Retrovirus particles produced by teratocarcinoma cells after induction. **a** and **b** Tera-1 cells; **c** and **d** GH cells; **e** and **f** HL cells. The *bars* represent 100 nm

Table 1. Effects of in vitro virus induction treatment of human teratocarcinoma cultures

Tumor	Morphology		Beta-HCG synthesis (m IU/ml)[a]		Virus production	
	Before induction	After induction	Before induction	After induction	Before induction	After induction
Tera-1	Monolayer	Domes, vesicles	0.7	25.6	+[b]	++
Tera-2	Monolayer	Monolayer	2.2	3.0	−	−
GH	Domes, vesicles	Domes, vesicles	10.8	21.2	+	+++
HL	Domes, vesicles	Domes, vesicles	52.3	100.2	−	+

[a] m IU/ml = Milli international units/ml. Background ≤ 5 mIU/ml
[b] Arbitrary judgement from electron microscopical observations

After the induction treatment not only a striking increase in virus production but also an alteration in cell morphology can be recognized. While uninduced cultures of Tera-1 show a monolayer of homogeneous cells, after induction several cell types arise within a matter of 3 to 4 days and dome and vesicle formation can be seen. The GH and HL cells, already selected for dome and vesicle formation, exhibit only limited morphologic alterations. In contrast, Tera-2 cells cannot be induced by chemical means to initiate virus production and cellular differentiation (see Table 1).

In the differentiated tumor cell populations the proportion of cells producing virus-like particles as judged by electron microscopy varies from a few in HL to over 10% in Tera-1 to almost 100% in GH cultures. The latter cell line, subcultivated only by transferring vesicles to new culture flasks, exhibits in any case a low spontaneous production of retrovirus particles. Thus it appears likely that virus replication is permitted only in a specific cell type of the differentiating culture.

In placentas it is the syncytiotrophoblast cell layer from which retrovirus particles can occasionally be seen budding (Kalter et al. 1973; Vernon et al. 1974). Syncytiotrophoblasts at the same time produce human chorionic gonadotropin (HCG) (Morisada et al. 1972). As a first step in determining the type of the virus-producing cell, we tested the secretion of HCG into the culture medium before and after the induction procedure (Table 1). While in Tera-1, GH, and HL cells HCG production after induction is significantly increased, this hormone is not detectable in the supernatant of Tera-2 cell cultures. Induction or enhancement of virus synthesis is thus paralleled by induction of HCG production. The alteration in hormone release may well reflect cellular differentiation, but it is not possible at present to claim that in analogy to the findings in human placentas, it is a trophoblastic or at least a HCG-secreting cell that is permissive for virus replication.

Attempts to further characterize the human teratocarcinoma derived viruses have so far been unsuccessful. Firstly, we were not able to demonstrate unambiguously reverse transcriptase (RT) activity either in uninduced or induced cultures. Transient small peaks of RT activity (Kurth et al. 1980) are observed but are difficult to interpret. The difficulty in detecting RT activity in the supernatant of teratocarcinoma cell cultures has also been reported by Bronson et al. (1978). Secondly, the teratocarcinoma viruses have not yet been demonstrated to be infectious. Cultivations between induced teratocarcinoma cells and a number of uninfected indicator cell lines did not yield replicating virus even after repeated cocultivations and passages for over 20 weeks. The indicator cells were derived from dog thymus (8155), bat lung (Tb-1-Lu), marmoset fibroblasts (HF), tupeia embryo kidney (TEK), mink lung (C58), and feline embryo fibroblasts (FEF), as well as from human cells (rhabdomyosarcoma RD, amnion cells AV-3).

C. Discussion

Whereas in uninduced human teratocarcinoma cells expression of retrovirus particles is a rare event (Bronson et al. 1978, 1979; Kurth et al. 1980), the production of such particles in three out of four teratocarcinoma cell lines can easily be demonstrated after treatment with IUdR, DXM, and DMSO. Individual tumor lines may also produce virus particles spontaneously.

The human teratocarcinoma derived particles differ morphologically from mammalian type-C viruses by the lack of a clear electron lucent space between core and envelope. In this regard they resemble the particles observed in human term placentas. The lack of easily demonstrable RT activity and infectivity as well as the absence of "mature" particles with condensed cores is also reminiscent of a number of retrovirus mutants (e.g., murine leukemia virus murants ts3 and ts24) (Witte and Baltimore 1978). These mutants are characterized by a defect in viral precursor protein processing. It is as yet largely unknown to what extent viral or host cell proteases contribute to the specific cleavage of virus precursor proteins to yield mature and infectious particles.

Therefore, the question cannot yet be decided whether the human teratocarcinoma-derived viruses posses an intrinsic genetic defect or whether the host cell is lacking a factor which is necessary for an appropriate processing of viral structural components. The latter possibility is somewhat favored by the recent findings of Gautsch (1980) that murine teratocarcinoma cells lack a factor needed to support the

replication of infecting murine leukemia (Teich et al. 1977) and papova (Swartzendruber and Lehman 1975) viruses.

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