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The Role of the Viral Genome in Oncogenesis[1]

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Introduction

It has been well known for some time that exposure of cells to a variety of viruses is followed by "transformation," and that suitable technics will detect differences between the transformed cells and cells of the parental type. Markers often used to follow such transformation are changes in karyotype, in morphology, in antigenic makeup, and in the acquisition of tumorigenic potential. Although it is known that at least a portion of the virus genome persists following virus transformation of cells, little is known of the state in which the genome of DNA-containing viruses exists in the tranformed cell, and much more experimentation will be required to delineate the information added to the cells during transformation and the viral genes required to effect and to maintain the transformed state.

Specific Virus-induced Antigens in Hamster Cells following Transformation by SV40

The original observation by Huebner and his colleagues (12) that sera from hamsters carrying tumors induced by adenoviruses or SV40 contain antibody against a new antigen in the transformed cells was quickly confirmed in many laboratories. These antigens were soon localized in the nuclei of the transformed cells and were subsequently found to be induced during the cytolytic cycle of the virus in simian cells (for review, see Reference 20). Thus, this tumor (T) antigen represented the *first* indication that transformation of the cells by these DNA viruses is accompanied by persistence of new information, since the progeny cells all synthesized new virus-specific antigens. It was therefore no surprise when virus-specific messenger RNA was found in the same cells (1, 7), and it is generally assumed that the continued presence of both the antigen and the virus-specific RNA is reasonable evidence that at least a portion of the virus genome must still exist in the transformed cells. Since the viral antigen is not synthesized by these cells, the supposition has been made that late events governed by the virus genome are either not transcribed or translated, or that in many instances, the genetic information required for late events has been lost before, during, or after transformation.

Another antigen found in these cells is the transplantation rejection antigen (9), which is also highly specific for the virus effecting transformation. This antigen is generally demonstrated by immunizing the animals with the virus and challenging them with cells transformed by the same virus. In general, animals thus immunized will reject challenge of these, but not other, transformed cells.

Tevethia *et al.* (24) discovered a new virus-specific antigen at the membrane of hamster cells transformed by SV40 that could be detected by the indirect membrane fluorescence technic. Antibody against this antigen is made in animals resisting challenge of transformed cells, and there is a possibility, unproved at the present time, that the surface (S) membrane antigen and the transplantation rejection antigens are related. Kluchareva *et al.* (15) have confirmed this work with SV40 and Irlin (14) has applied the findings to the polyoma system where he appears to have obtained similar results with transformed mouse cells. Information available about these 4 antigens is summarized in Table 1 and reflects the work of many investigators.

A number of hamster cell lines developed by Diamandopoulos and Enders (3) following exposure to SV40 were shown by them to be devoid of the T antigen. It was of some interest to determine whether such cells also contain the S antigen and whether presence of this antigen could be related to oncogenicity. This study, carried out by Diamandopoulos *et al.* (4), demonstrated that hamster embryo fibroblast cultures exposed to SV40 varied in T and S antigen content following transformation. A number of such cultures synthesized both the T and S antigen, while other cultures synthesized only the S antigen. Presence of S antigen appeared to be correlated in these cells with oncogenicity for weanling hamsters (Table 2). As might be expected, a number of the cultures exposed to SV40 do not appear to have been transformed and contained neither the T nor S antigens; they were not oncogenic when inoculated into weanling hamsters. These results indicate that the synthesis of T antigen is not required for oncogenicity and that the S antigen is probably a better marker for this property than is the persistence of the T antigen. However, a number of control cultures not exposed to SV40 also became oncogenic; cells in these cultures were negative for the SV40 S antigen. Nevertheless, the fact that the S antigen is synthesized at the membrane of the cell and that the membrane may play a role in oncogenicity is also significant, although a direct relationship is lacking at the present time.

The possibility remained that the S antigen is a hamster cell antigen that increases quantitatively during or after transformation. Although remote, this possibility existed since antibody against this antigen was produced by inoculating animals with the cells themselves following immunization with the virus. Recently, antibody against this antigen has been produced without resort to challenge with viable transformed cells. This antibody has been obtained by inoculating newborn

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Table 1

Property	Virus-specific antigens			
	Tumor	Capsid	Membrane	Transplantation
Location	Nucleus	Nucleus	Surface	Surface
Productive cycle	Present	Present	Not known	Not known
Transformation	Present	Absent	Present	Present
Role in tumor rejection	None	None	Not known	Important
Source of antibody	Tumor-bearing hamsters	Immune animals	Tumor-rejecting hamsters	
Nature of antibody	7 S	7 S	Not known	

Comparison of properties of SV40-specific antigens.

Table 2

Culture	SV40 antigens		Oncogenicity
	T	S	
1	+	+	+
2	+	+	+
3	0	+	+
4	0	+	+
5	0	0	0
6	0	0	0
7	0	0	0

Antigenicity and oncogenicity of hamster embryo fibroblast cultures exposed to SV40.

hamsters with SV40 virus and then reinoculating them during the latent period of viral tumorigenesis (2, 5). The animals thus protected have been shown to develop antibody capable of reacting with the S antigen in the transformed cells (15, 23).

Recently, using a method devised by Harris and Watkins (10) to fuse cells of different species, small amounts of SV40 virus have been extracted from cells transformed by the virus (16; Dulbecco, personal communication). The liberation of minute quantities of virus from some transformed cultures had been shown earlier by Gerber (8) and by Sabin and Koch (22), but quantitative technics will have to be employed before the full significance of these observations can be determined.

Oncogenicity of Adenoviruses Carrying Defective SV40 Genetic Information

The biologic properties of the human adenovirus type 7 that has incorporated defective SV40 genetic material within its adenocapsid have been extensively studied and reviewed (20). The particle containing the defective SV40 genome is known as PARA. Its presence confers new properties on the adenovirus population, which now replicates in simian cells and carries genetic information for the synthesis of SV40 T antigen and SV40 specific transplantation antigens. The original adenovirus type 7 carrying PARA was shown to be highly oncogenic in newborn hamsters (11).

The SV40 information in the PARA particle has been transferred from the original parent adenovirus type 7 to other human adenoviruses by a process called transcapsidation (17). As a result of such transfer to adenovirus type 2, this previously nononcogenic adenovirus produced tumors when injected into newborn hamsters (18, 21). These tumors were characteristic of either SV40 induced tumors, adenovirus-like tumors, or they contained elements of both types (13, 21). Thus, it was expected that the transfer of the SV40 genome to other human adenovirus serotypes would result in the acquisition of oncogenic properties by those viruses.

Various PARA-adenovirus populations were therefore produced and were injected subcutaneously into groups of newborn hamsters within 24 hours after birth. In addition, other groups of newborn hamsters were injected subcutaneously with the parent adenovirus populations not carrying information in the PARA particle. The PARA-adenovirus types 1, 2, 5, and 6 become highly oncogenic, although the parent serotypes are nononcogenic (19). These adenoviruses contain DNA with a high (56–60%) guanine plus cytosine content and have thus far failed to produce tumors in newborn animals. However, recently Freeman *et al.* (6) have described the *in vitro* transformation of rat embryo cells with adenovirus type 2; these cells do not appear to be oncogenic when inoculated into rats, however.

Surprisingly, transfer of the SV40 genetic information to adenovirus types 3, 14, 16, and 21 failed to enhance the oncogenic potential of these viruses (19). These are adenoviruses with an intermediate (50–53%) guanine plus cytosine in the viral DNA, and the parent serotypes are "weakly" oncogenic when inoculated into newborn hamsters. The reason for failure of these viruses to become more oncogenic when the known oncogenic markers of SV40 are added is unknown at the present time. It is perhaps important to note that all adenovirus serotypes carrying PARA are able to induce resistance to the transplantation of cells transformed by SV40 (18, 19). These results suggest that the virus is able to enter the hamster cell to induce the transplantation rejection antigen, which makes even more surprising the fact that such entry does not result in transformation.

These results clearly reveal that only certain adenoviruses become oncogenic following the acquisition of SV40 determinants. Though we are unable to explain these results at the present time, they indicate that many advances must still be forthcoming before we can hope to understand the molecular events governing the transformation of cells by animal viruses.

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