A specific signature of Merkel cell polyomavirus persistence in human cancer cells

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Presently, slightly >20% of the global cancer incidence has been linked to viral, bacterial, and parasitic infections (1). Some of these agents act as direct carcinogens, where persistence and expression of viral oncogenes are required to maintain the malignant phenotype of the cancer cells; others act indirectly, e.g., by inducing immunosuppression or chronic inflammation or activating oxygen- or nitroso-radicals. Commonly, in direct carcinogenesis the genomes of oncogenic viruses persist, being either integrated into host cell DNA or as episomes within the cancer cells. Under specific conditions viral DNA may become reactivated (Epstein–Barr virus, human T lymphotropic retrovirus, human herpesvirus type 8), resulting in complete cycles of viral replication. High-risk human papillomaviruses often acquire partial deletions of their genomes in the course of viral integration into host cell DNA, and some of the persisting Epstein–Barr virus genomes may become replication-defective in Burkitt's lymphoma cells. In hepatitis B virus-linked carcinogenesis integrated viral DNA fragments may persist in an apparently random pattern.

Merkel Cell Polyomavirus

In this issue of PNAS, Shuda *et al.* (2) report a more detailed analysis of Merkel cell polyomavirus (MCPyV) genomes in Merkel cell carcinomas, following up their initial observations of the frequent presence of this viral DNA in Merkel cell tumors (3), confirmed already in other laboratories (4, 5). Nine Merkel cell carcinomas containing MCPyV DNA all were shown to harbor mutations prematurely truncating the MCPyV large T antigen (LT) helicase. These mutations were absent in 4 MVPyV isolates from nontumor sources, whereas in the tumor tissues the viral DNA persisted in an episomal form. The mutations left the Rb-binding domain intact. The availability of a Merkel carcinoma cell line (MKL-2) containing MCPyV DNA with a monoclonal integration pattern permitted functional tests of the tumor-derived and the wild-type LT. Only wild-type LT activated replication of integrated MVPyV DNA in the tumor cell line, whereas mutated LT was replication-deficient. Shuda *et al.* found a high rate of pyrimidine dimer substitutions among LT mutations. In view of the suspected role of UV light in Merkel cell tumorigenesis (6, 7), they proposed as a model for the evolution of Merkel carcinomas the initial integration of viral DNA, followed by mutations abolishing the helicase function, but leaving the Rb-binding functional domain intact. They interpret these data as a virtual exclusion for a passenger role of MCPyV in these carcinomas.

Transformation by Replication-Deficient Viruses

Mutations within a specific region of viral oncogenes as a possible prerequisite for human carcinogenesis have not yet been reported and emerge as a novel mechanism by which infections may contribute to human cancers. The mutations reported by Shuda *et al.* (2) occur within the helicase part of the large T antigen of MCpyV and result in replication incompetence. This finding is reminiscent of early observations made by Gluzman and colleagues (8–10). That group reported that replication-defective SV40 virus DNA revealed an elevated transformation potential for various cell types. SV40 T antigen mutants defective for origin-binding possess supertransforming properties (11). Subsequently, similar observations have been reported for large T antigen-defective polyoma viruses (12) and the transforming transmembrane protein of the jaagsiekte virus after introduction of specific mutations (13). Thus, replication-defective polyoma viruses with even enhanced transformation potential arose from the loss of T antigen binding to the origin of replication. Conversely, modifications of the origin of replication, no longer permitting T antigen binding, are expected to have a similar effect.

Polyomavirus infections are commonly nononcogenic for their natural hosts.

As far as the Merkel cell polyomavirus is concerned, we are obviously only at the beginning in understanding its complex interactions with the human host. It will be important to identify its permissive cells within the human host, if they exist. The virus has now been discovered in a number of nonmalignant human cells, apparently persisting there in episomal form without T antigen mutations, and retains its replicative potential. Because most of the MCPyV-positive normal tissues were derived from skin biopsies (4), specific cutaneous cells may represent sites of virus replication. Indeed, unidentified polyomavirus-like particles have been repeatedly reported in trichodysplasia-related conditions of human skin (reviewed in ref. 13).

Another important aspect concerns the immortalizing and transforming activities and thus the function of replication-defective and replication-competent T antigens of MCPyV. Does the replication-defective T antigen selectively stimulate growth in neuroendocrine cells, from which Merkel cell carcinomas are derived, and spare other types of human cells? The implication of Shuda *et al.* (2) that integration of viral DNA and the mutagenic function of UV exposure targeting the helicase part of T antigen act as triggering events may be persuasive. It fails, however, to explain the relative rarity of Merkel carcinomas, the commonly high age of tumor incidence, and their increased frequency under conditions of immunosuppression. It seems to be very likely that additional modifications, probably of host cell genes, are required for malignant conversion.

As an interesting aspect, polyomavirus infections are commonly nononcogenic for their natural hosts, which also accounts for the human polyomaviruses BK and JC. Evidence for their possible role in human cancers is at best scarce and remains controversial (2), although they are widely spread in all human populations. The mechanism of this host-specific protection is not well un-

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derstood. Obviously, this protection originates from a long coevolution of these viruses with their natural hosts. Their apparent nonpermissiveness for some animal species seems to be 1 precondition for oncogenic function of their tumor antigens, which should raise the question of whether MCPyV adapted relatively recently to the human host. In this case it is reasonable to assume that it crossed the species barrier originally from animal hosts that are in close contact with humans.

It has been hypothesized previously that tumorvirus infections of domestic animals, nononcogenic in their natural hosts, may result in nonpermissive infections of humans, where the genomes, now replication-defective for the human cells, may still exert growth-stimulating functions (14, 15). If not integrated into specific chromosomal sites and present and persistent in low copy numbers their detection may prove to be exceedingly difficult.

It seems to be predictable that the discovery of MCPyV and its likely role in a substantial percentage of Merkel cell carcinomas and its peculiar modifications of large T antigens in malignant tumors opens an exciting new chapter in human tumor virology, guiding the path for future explorations.

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