Herpesvirus and the Lucké Tumor

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Summary

*Rana pipiens* is host to a renal adenocarcinoma (Lucké tumor) that occurs with high frequency in a wild heterogeneous animal population. There is a relationship between temperature and the presence or absence of herpesvirus in tumor cells. Tumor cells of frogs either in hibernation or maintained at low temperature (4 to 9°) in the laboratory contain intranuclear inclusion bodies (Cowdry type A) and herpesvirus. In contrast, neither inclusions nor virus are found in tumor cells of frogs captured in the spring or summer or maintained in the laboratory at 20 to 25°. Lucké tumors can be induced in developing frog embryos by cell-free tumor extracts containing herpesvirus or by ascitic fluid containing this virus. A number of cell cultures (from insect to mammalian) have been tested for susceptibility to the Lucké tumor herpesvirus, but none has been found that will support multiplication of the virus. A number of other viruses have been isolated, including a herpesvirus distinct from the one seen in tumor cells, but none of them induce tumors.

Introduction

A relationship between cervical carcinoma of humans and renal carcinoma of frogs may seem remote. The fact is, however, that each of these cancers, regardless of host, may be induced by a herpesvirus, thereby providing a basis for bringing together information on cancer involving 2 hosts so widely separated phylogenetically. There are similarities between the 2 systems; both are carcinomas rather than lymphoproliferative tumors or sarcomas, as are most virus-induced neoplasms, and both depend on cell physiology as influenced by temperature (6) or pH (1) for the presence or absence of virus in tumor cells. Thus, we may predict that information gained from an understanding of the natural history of the Lucké tumor, particularly the role of a herpesvirus in its evolution, will contribute to an understanding of the role of herpesvirus, not only in cervical carcinoma but in other candidate herpesvirus tumors of humans as well.

The following is a brief review of the state of information and knowledge regarding the Lucké tumor. For more detailed and illustrated accounts of this subject, the reader should consult recent reviews (5, 6).

Characteristics of the Lucké Tumor

The Lucké tumor, a renal adenocarcinoma of *R. pipiens*, is very frequently found in wild heterogeneous frog populations of the northeast and north-central parts of the United States. The incidence of gross tumors can be as high as 10% in frogs captured in nature (12, 16) and may approach 50% among large adults held in the laboratory at 25° for a long period of time (22). A recent study, which included histological examination of serial sections of apparently normal frog kidneys as well as of gross tumors, suggests an incidence approaching 100% (15). These results, if confirmed, will be of fundamental importance in an evaluation of the natural history of the disease and its viral etiology.

Cells of tumors from overwintering frogs or from frogs held at 4 to 9° in the laboratory (hereafter referred to as cold tumors) contain Cowdry type A intranuclear inclusions. The presence of nuclear inclusions is invariably correlated with the presence of a herpesvirus (3, 14) that can be physically extracted (13, 19). The frequency of virus-containing tumor cells may vary from as low as 1% or less to almost 100%. The cells containing virus are degenerating cells, indicating that the presence of virions is incompatible with cell survival. Urine or ascitic fluid from frogs bearing cold tumors may also contain herpesvirus (7, 20).

In contrast to cold-tumor cells, tumor cells of frogs either captured during the warm months or held in the laboratory at 20 to 25° (hereafter referred to as warm tumors) do not contain intranuclear inclusions or virus. When frogs bearing warm tumors are held at 4-9° for 1 to 7 months, intranuclear inclusions are found in some of the tumor cells (21, 26). Conversely, when frogs bearing cold, virus-containing tumors are held at 20 to 22°, tumor cells are virus free within 7 days (28).

The observations on the effect of temperature on the presence or absence of virus in *in situ* tumors have been confirmed with 2 experimental techniques, *i.e.*, by transplantation of tumor fragments to the anterior eye chamber of frogs followed by temperature shift (17, 18) and by explants of tumor fragments in tissue culture (2, 23). The tissue culture experiments are important because they show that the intact host is not required for induction of virus production at low temperature.

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1 Presented at the American Cancer Society Conference on Herpesvirus and Cervical Cancer, December 8 to 10, 1972, Key Biscayne, Fla. This study was supported by USPHS Research Grant CA 07055, Childhood Cancer Research Center Grant CA 08480, and Contract NIH 71-2134 from the National Cancer Institute, by Grant DRG 1073 from the Damon Runyon Memorial Fund for Cancer Research, and by ALSAC.
Properties of the Herpesvirus Extracted from Lucké Tumors

Relatively little information is available on the physical, chemical, and biological properties of the herpesvirus (LHV)² physically separable from the Lucké tumor. The DNA of LHV is a linear double-stranded molecule with a base composition of 45 to 47% guanine plus cytosine (9, 27). There appears to be nothing distinctive in virus ultrastructure or in development of the virus in tumor cells as reconstructed from electron micrographs (14, 24). As is discussed below, crude or semipurified preparations of LHV obtained from cold tumors induce typical Lucké tumors in developing frog embryos (25). Tumor-inducing activity is destroyed by protease or by lyophilization but is stable to sonication (26).

Transmission Experiments

Although the cell-free transmission experiments of Lucké (11) provided evidence for the viral etiology of the tumor named after him, the 1st truly significant experiments were reported by Tweedell (25). He conclusively demonstrated that R. pipiens embryos or larvae that were given injections of cytoplasmic fractions of cold tumors and held at 20° developed virus-free renal tumors as they reached metamorphosis. The inoculum contained herpesvirus, as determined by electron microscopy, and filtrates retained activity. Comparable fractions of normal, adult frog kidneys or of warm tumors did not induce tumors. Preparations containing enveloped LHV obtained by rate-zonal centrifugation of cytoplasmic fractions of cold tumors have since been shown to be oncogenic (17), as has herpesvirus-containing ascitic fluid from a frog bearing a cold tumor (20). When froglets bearing induced tumors are kept at 9°, intranuclear inclusions appear in some tumor cells (26). These transmission experiments clearly demonstrate that the Lucké tumor is virus induced and that a herpesvirus almost certainly plays a role in its etiology. The reason I say “almost certainly” is because viruses other than LHV are present in Lucké tumors (8). Although none has induced tumors, the presence of such viruses, coupled with the inability to isolate LHV in tissue culture (see “Attempts to Isolate LHV”) requires this modest degree of reservation concerning LHV as the sole causative agent of the Lucké tumor.

Attempts to Isolate LHV

Several viruses unrelated to the herpesvirus group have been isolated from Lucké tumors and have been propagated in vitro. They do not induce tumors, and their properties have been reviewed recently (4). Of greater interest was the isolation by Rafferty (21) of a virus from the pooled urine of cold-tumor-bearing frogs that produced a cytopathology similar to herpesvirus. Subsequently, the virus was identified as a herpesvirus (10), but it was distinguishable from

³The abbreviation used is: LHV, Lucké herpesvirus.

<table>
<thead>
<tr>
<th>Source</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insect</td>
<td>Mosquito: cabbage looper</td>
</tr>
<tr>
<td>Fish</td>
<td>Brown bullhead; fathead minnow; blue gill; rainbow trout (gonad)</td>
</tr>
<tr>
<td>Amphibian</td>
<td>African clawed toad (liver or kidney); newt (iris); leopard frog (embryo, adult kidney, Lucké tumor); spring peeper (embryo); toad (kidney)</td>
</tr>
<tr>
<td>Reptile</td>
<td>Lizard (iguana heart, gekko lung); turtle (heart); viper (heart)</td>
</tr>
<tr>
<td>Mammalian</td>
<td>Baby hamster (kidney)</td>
</tr>
</tbody>
</table>

LHV by a number of criteria (9) and did not induce tumors (8). Thus R. pipiens may be host to at least 2 different herpesviruses.

Naegle and Granoff (unpublished data) have intensified the search for an in vitro cell system capable of supporting multiplication of LHV. A variety of cells, from insect to mammalian (Table 1), have been exposed at both 7.5 and 25° for 3 to 5 months to subcellular cold-tumor fractions containing LHV. No evidence of virus replication as determined by cytopathology, cell transformation, or electron microscopy has been obtained.

Conclusion

The Lucké tumor provides an opportunity to study the natural history of a high-frequency, spontaneously occurring viral tumor and offers the additional opportunities of a broad range of experimental design not possible with other virus tumor systems. Significant advances await the isolation and propagation of the etiological agent in tissue culture. When this is accomplished, this experimental model may provide new and relevant information on viral carcinogenesis in humans.

References

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