Studies of Hamster-Specific Oncogenic Virus Derived from Hamster Tumors Induced by Kirsten Murine Sarcoma Virus (34551)

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Kirsten *et al.* (1–2) described the isolation of a murine sarcoma virus (MSV) from a rat passaged murine erythroblastosis virus (3). The Kirsten MSV (Ki-MSV) shares the group-specific complement-fixing (CF) antigens of the murine leukemia-sarcoma virus group (4) and is similar to the Moloney (5) and Harvey (6) strains of MSV in its capacity to produce sarcomas in the heterologous hamster host (7).

The Ki-MSV hamster tumor cells contain the MSV genome demonstrable in virus rescue techniques with helper leukemia virus (8, 9) but contain no detectable viral CF antigens or infectious virus capable of replicating in mouse embryo fibroblast (MEF) cultures (4, 7). However, Klement *et al.* (7) recently found that such tumors contained an antigenic and host-range variant virus which was oncogenic only for the hamster species both *in vivo* and *in vitro*. Recent independent observations of other investigators also indicate the existence of such hamster-specific sarcoma viruses in hamster tumors induced by other murine sarcoma viruses such as Harvey strain of MSV (10), Moloney MSV (11) and the Gross pseudotype of this virus (12).

In our independent studies reported here, we made several isolations of hamster-specific sarcomagenic and focus-forming viruses from hamster tumors induced by the Ki-MSV. In addition, we found that a Ki-MSV derived hamster-specific sarcoma virus isolate established a virus productive carrier state in hamster cells transformed by this virus. Virus derived from such transformed cultures induced hamster cell transformation *in vitro* and progressively growing sarcomas in newborn hamsters.

Methods. Experimental animals used in our experiments were procured from the National Institutes of Health. Tissue culture techniques used have been described (8, 13, 14). A hamster bearing the third subcutaneous transplant passage of Ki-MSV hamster tumor was kindly provided by Dr. V. Klement. A tumor transplant line was established in weanling Golden Syrian hamsters.

Using tumors at the third, fourth, and fifth transplant passages, partially purified virus concentrates were prepared by a modification (9) of the technique described by Moloney (15). These preparations were used either immediately or after storage at —70°. These preparations were inoculated in parallel into newborn hamsters, newborn NIH Swiss and $DBA/_2$ mice and into secondary cultures of NIH Swiss and $DBA/_2$ mouse embryo fibroblasts and into hamster embryo fibroblasts (HEF).

The inoculated animals were observed for tumor development. When tumors appeared, they were serially transplanted into newborn hosts of homologous species. Clarified tumor extracts were tested for reactivity in CF test vs. murine leukemia group-specific CF antibodies prepared in rats (4). The presence of infectious virus was tested by inoculating partially purified tumor concentrates (9, 15) into newborn hosts and into tissue cultures as described above.

Inoculated tissue cultures were observed for foci of cell transformation. The cultures were maintained and propagated by serial transfer of cells. Several cell lines of normal and transformed cultures were established. Cell antigens of inoculated and control cultures were tested for the presence of murine leukemia group-specific CF antigens (4). Clarified cell-free culture fluids (filtered

TABLE I. Hamster Specificity of Sarcomagenic and Focus-Forming Virus Recovered from Kirsten MSV Hamster Tumors and Its Serial Propagation in Hamster Species.

Material inoculated	Induction of progressive sarcoma *in vivo*			Transformation of cells *in vitro*[a]			
	NIH Swiss mice	$DBA/_2$ mice	Hamsters	NIH Swiss mouse	$DBA/_2$ mouse	NRK rat	Hamster
Kirsten MSV hamster[b] sarcoma	0/16	0/12	14/14	—	—	—	+
Hamster-specific virus[b] induced hamster sarcoma	0/15	0/16	13/13	—	—	—	+
Cell-free culture medium of hamster cells transformed *in vitro* by hamster-specific virus (Line SLH)	0/10	0/13	11/11	—	—	—	+

[a] + = positive for cell transformation; — = negative for cell transformation.
[b] Partially purified cell-free preparations (9, 15).

through Millipore 0.45 μ filters to remove cells) were tested for infectious virus by the procedures described above.

Ultrathin sections of an established line of transformed hamster embryo culture (Line SLH) were examined for the presence of virus particles. Virus production was also determined by labeling cultures with tritiated uridine and assaying supernatant fluids for newly synthesized virus (16). A monolayer culture of cells was incubated for 48 hr at 37° with 20 ml of medium (Eagle's basal medium with 10% fetal bovine serum) containing 20 μCi/ml of ^3H uridine (25.2 Ci/mmole). The supernatant fluid was decanted and freed of cells by centrifugation at 10,000 *g* for 20 min. Fifteen ml of fluid was then centrifuged in a 15–60% sucrose gradient at 24,000 rpm for 3 hr in the Spinco SW 25.1 rotor. The tubes contained 7 ml of 10% sucrose solution between sample and gradient. Fractions were collected by puncture of the bottom and density was determined from the refractive index (Abbe refractometer) based on values for known sucrose concentrations. Fractions were collected on Millipore filters after precipitation with 10% trichloroacetic acid and radioactivity was determined in a Nuclear-Chicago ambient temperature scintillation counter.

Results. Hamsters inoculated subcutaneously on the day of birth with partially purified preparations of Ki-MSV hamster tumors developed progressively growing fibrosarcomas within 15 to 20 days of inoculation. Representative results of several *in vivo* and *in vitro* experiments are shown in Table I. The tumors were cystic and hemorrhagic. Unless sacrificed, death occurred within 40 to 50 days of inoculation.

Newborn NIH Swiss mice and $DBA/_2$ mice, which were similarly inoculated in parallel, either failed to develop tumors or developed subcutaneous nodules of less than 10 mm which rapidly regressed. Attempts to transplant these nodules into newborn mice were unsuccessful.

The induced hamster tumors were serially transplantable in newborn and weanling hamsters but not in $DBA/_2$ or NIH Swiss mice. The transplanted hamster tumors retained the hemorrhagic, cystic character. Partially purified preparations of these tumors, in turn, were tumorigenic in hamsters but not in NIH Swiss or $DBA/_2$ mice.

The partially purified tumor preparations induced foci of cell transformation in secondary monolayer cultures of hamster embryo fibroblasts but not in NIH Swiss or $DBA/_2$ MEF cultures or NRK strain of rat kidney cells (17) (Table I). The foci consisted of aggregates of refractile spindle cells. Generally 10 to 20 foci were observed within 7 days of inoculation of each 60-mm culture dish

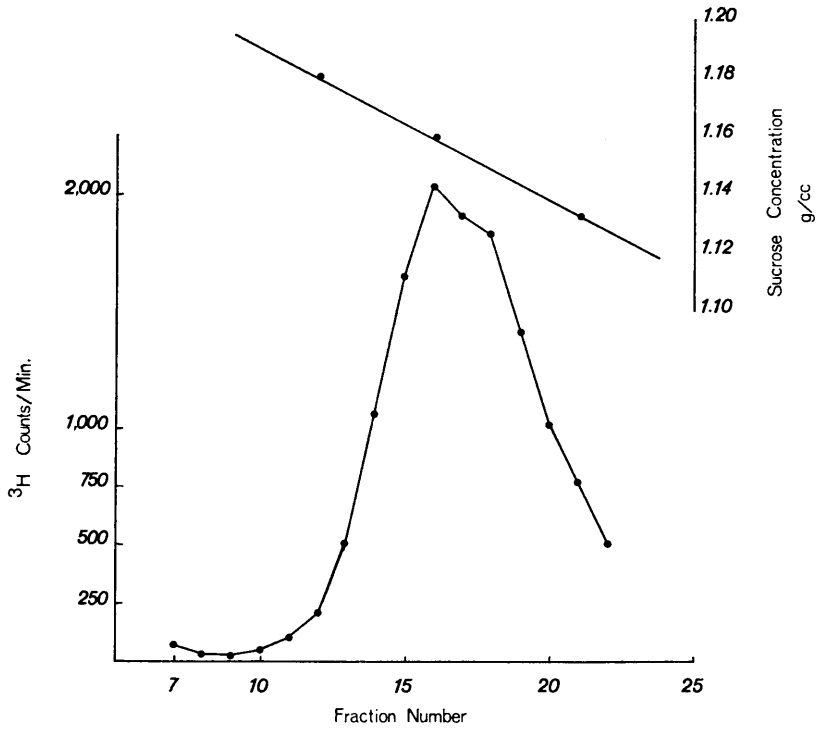

FIG. 1. Virus production by SLH hamster tumor cells. A radioactive peak 100 times the background radioactivity was obtained at 1.16 g/cc in sucrose gradients.

indicating the presence of 50 to 100 hamster cell transforming units of virus per milliliter of the hamster tumor preparations.

On serial transfer of cultures, some cultures lost the transformed morphological characteristics, while in others, transformed cells predominated over normal cells ultimately resulting in the establishment of cell culture lines of transformed cells.

A transformed culture designated SLH was studied in detail. The cells were oncogenic for newborn and weanling hamsters but not for NIH Swiss or DBA/$_2$ mice (Table I). Clarified culture fluids (filtered through Millipore 0.45 μ filters to remove cells) readily induced progressively growing tumors in all inoculated newborn hamsters and foci in hamster embryo cultures, but failed to elicit a tumor response in newborn NIH Swiss or DBA/$_2$ mice or to induce foci in embryo cultures of these species and NRK rat cells (17) (Table I).

The SLH line of transformed hamster cells were found to contain numerous mature and budding C-type virus particles. The release of the oncogenic RNA tumor virus from the transformed cells was readily detected by labeling cultures with tritiated uridine and assaying supernatant fluids for newly synthesized virus (Fig. 1). A radioactive peak 100 times the background radioactivity, was obtained at 1.16 g/ml in sucrose gradients.

A CF antigen similar or identical to the murine leukemia group-specific CF antigen (4) was not demonstrable in hamster tumors (20% clarified extracts, partially purified preparations) and cultured tumor cells or cells transformed *in vitro* by the hamster-specific sarcoma virus (20% cell packs, disrupted cells). Similarly, cultures of MEF and NRK cells (17) which were tested for ability to replicate the hamster-specific virus also did not develop a CF antigen, reactive in the COMuL test indicating the absence of viruses of the murine leukemia-sarcoma group (4). Antisera prepared in rats against Gross mur-

ine leukemia virus and M-MSV failed to neutralize the focus-forming effect of Ki-MSV hamster-specific virus (7).

Discussion. Ting (17) reported the isolation of a NRK rat cell-specific sarcoma virus from Moloney strain MSV-induced rat tumors. The virus was antigenically distinct from Moloney MSV. He designated this virus as MSV(0) since the virus appeared to have no associated helper murine leukemia virus. Similarly, the Ki-MSV derived hamster-specific virus reported by Klement *et al.* (7) and the similar virus we isolated appear to be specific for hamster cells but there is no definite proof that the virus is without a helper leukemia virus. The hamster-specific virus may be distinct from NRK rat cell-specific MSV(0) since it failed to induce foci in cultures of NRK cells. In addition, rat antiserum prepared against MSV(0) failed to neutralize the Ki-MSV hamster-specific sarcoma virus. The recovery of antigenic and host range variants from MSV-induced hamster and rat tumors appears to be similar to the recovery of RSV(0) from chicken nonproducer cells (18, 19).

The mechanism involved in the modification of the antigenic and host-range characteristics of Ki-MSV by passage through hamsters is unknown and remains to be determined. One possibility is that passage through resistant hamster hosts resulted in a selection of an antigenic variant capable of replicating in hamster hosts. This is suggested by observations on antigenic shifts in avian leukosis and sarcoma viruses when virus stocks containing antigenic mixtures are passaged through host cells selectively resistant to one or more antigenic types of the virus (20–22). It is also possible that the hamster-specific virus is a pseudotype of Ki-MSV with the envelope of an indigenous hamster leukemia virus (7, 9, 12).

In the studies of Klement *et al.* (7), it was found that the Ki-MSV derived hamster-specific sarcoma virus induced morphological transformation of hamster cells *in vitro* but the virus failed to replicate to form infectious virus. In the studies reported here, we found that the SLH line of transformed hamster cells, originally transformed by the Ki-MSV hamster-specific sarcoma virus, released sarcomagenic and focus-forming virus similar to the hamster specific virus-producing cell line derived from a Harvey MSV hamster tumor (10).

Our studies and independent studies of other laboratories (7, 10–12) thus indicate the existence of hamster-specific sarcomagenic and focus-forming viruses in MSV hamster tumors which were previously considered to be free of infectious virus. Further studies are necessary to establish the antigenic, biological and biophysical characteristics and the interrelationships of the various MSV-derived hamster-specific sarcoma virus isolates and the recently reported hamster lymphoma virus (23).

Summary. Hamster tumors induced by the Kirsten strain of murine sarcoma virus (Ki-MSV) were found to contain a virus antigenically distinct from Ki-MSV. The virus had a restricted host-range and was infectious only for the hamster species but not for the mouse species from which the Ki-MSV was originally derived. The hamster-specific virus produced foci of cell transformation in hamster embryo fibroblasts and progressively growing sarcomas in newborn hamsters. One established tissue culture line of *in vitro* transformed hamster embryo cells (Line SLH) was found to release the hamster-specific focus-forming and sarcomagenic virus. These independent observations confirm and extend the recent observations of other investigators on the presence of such hamster-specific viruses in hamster tumors induced by various strains of murine sarcoma virus.

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