Deletion Mutations in the Small t Antigen Gene Alter the Tissue Specificity of Tumors Induced by Simian Virus 40

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Subcutaneous injection of wild-type simian virus 40 into Syrian hamsters normally induces fibrosarcomas at the injection site. We showed that subcutaneous injection of three different small t deletion mutants (dl884, dl883, and dl890) led to the formation of abdominal reticulum cell sarcomas (lymphomas) in about 15% of the animals bearing tumors. The remainder of the tumors were fibrosarcomas occurring with prolonged latencies at the site of injection. We postulated that, in the absence of an active small t protein, which is thought to have cell growth-promoting properties, the mutant virus preferentially transforms rapidly proliferating lymphoid cells.

Simian virus 40 (SV40) is highly oncogenic in hamsters (5). Newborn animals are particularly susceptible, usually developing fibrosarcomas at the injection site within 6 months after subcutaneous (s.c.) inoculation of the virus (6-8). The SV40 large T and small t antigens encoded by the early region of the virus are responsible for in vivo oncogenesis as well as in vitro cell transformation (18). To understand the roles that these viral proteins play in the loss of growth control involved in cellular transformation, transformation-defective mutants of SV40 have been isolated and studied (1, 10, 15, 16). SV40 mutants with deletions in the small t antigen gene are of particular interest since they appear to be defective in transformation of rodent cells in culture only under certain conditions (e.g., when cells are growth inhibited at the time of infection) (12, 13). When injected s.c. into newborn hamsters, small t deletion mutants induced s.c. fibrosarcomas with significantly prolonged latency periods (4, 9, 19). In addition, studies in our own laboratory indicated that, in some of the animals inoculated s.c. with one small t deletion mutant (dl884), tumor foci developed at sites distant from the site of injection (4). In some cases, these distant tumor foci appeared to arise as metasteses from primary s.c. fibrosarcomas, whereas in others, tumors were present only in the viscera and not at the s.c. site of injection.

To determine whether these altered tumorigenic properties arise from small t antigen lesions in general or whether our observations pertained uniquely to the dl884 mutant, we expanded our studies to include additional mutants that are deleted in the small t antigen gene. Four small t deletion mutants (dl884 [4502 to 4748 deleted] [17]; dl883 [4532 to 4588]; dl890 [4720 to 4746]; dl2006 [4560 to 4748] [15]) and wild-type 830 (wt830; the parent of the 800 series deletion mutants [14]) were injected s.c. between the scapulae of groups of newborn random-bred Syrian hamsters (within 48 h of birth). Typical profiles of tumor appearance are shown in Fig. 1. As noted previously (4, 9, 19), tumors induced by the deletion mutant viruses developed much more slowly (average mean latency, 59 weeks) than wild-type virus-induced tumors (average mean latency, 35 weeks). The latencies of wt830- and dl884-induced tumors were largely unaffected by the total dose of virus over a 500-fold range (4 $\times$ 10$^5$ to 2 $\times$ 10$^6$ PFU per animal).

In confirmation of our previous studies (4), we observed virus-specific differences in the sites of tumor appearance. Animals injected with wild-type virus bore firm s.c. fibrosarcomas (or undifferentiated sarcomas; Fig. 2A) near the site of injection. A similar pathologic picture was found in many animals injected with the deletion mutant viruses. However, among animals bearing dl884-, dl883-, and dl890-induced tumors, 15% developed histologically different tumors, which we identified on morphologic grounds as reticulum cell sarcoma (referred to here as lymphoma, according to human histopathologic terminology; Fig. 2B). In these animals, the tumors were present in the abdominal cavity (within the mesenteric and peritoneal lymph nodes, the folds of the intestines, the spleen, and the liver); the mediastinal nodes were frequently tumorous as well. In contrast to the deletion mutant-induced s.c. tumors, the visceral tumors appeared with a mean latency comparable to that of the wild-type virus-induced s.c. tumors (Table 1). None of the animals that had visceral tumors bore detectable s.c. fibrosarcomas. Tumor foci were found in lungs (presumably due to metastasis) with equal frequency (about 5%) in animals bearing s.c. fibrosarcomas induced by either wt830 or the small t deletion mutants, but lung nodules were present in almost all of the animals with lymphoma.

Of 130 animals injected with wt830, only one developed an abdominal tumor identified as a reticulum cell sarcoma. However, the latency of this tumor was about double the mean latency for most wild-type virus-induced tumors, and we have no evidence as to whether it was virus induced. Among 41 animals injected with the dl2006 virus, no abdominal tumors were observed. However, in 2 of 22 tumor-bearing animals, osteogenic sarcomas occurred. The wild-type parent (strain 776) of the dl2006 mutant was not included in this study, so the significance of this observation is not clear. Moreover, DNA sequencing in our laboratory revealed that the dl2006 mutant, and not its 776 parent, has a 21-base-pair insertion (sense strand: 5'-CTAACTG ACACACATTCCACA-3') between positions 178 and 179 of

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the viral genome at the junction between the 72-base-pair repeats of the transcriptional enhancer.

To study the characteristics of the deletion mutant-induced tumors more completely, we established cultured cell lines from both s.c. fibrosarcomas and abdominal reticulum cell sarcomas. Cells from the fibrosarcomas grew in culture with a characteristic fibroblastic appearance; in contrast, cell lines established from the abdominal tumors were made up of rounded, loosely adherent, and floating cells. Cells from both types of tumors stained positively for nuclear SV40 T antigen and formed tumors when reinjected into adult hamsters. Three abdominal tumor cell lines (representing the three 800 series deletion mutants) and several fibrosarcoma cell lines were chosen for further study. Analysis of the integrated SV40 viral sequences by Southern blotting revealed no striking differences in viral integration patterns in cells from the two types of tumors. SV40 could be rescued from both types of cells, and the viral DNA was shown by restriction analysis to be identical to that of the injected virus. In addition, by DNA sequence analysis, we showed that 800 series input and rescued viruses were identical to each other in the transcriptional enhancer region. This latter observation indicated that the alteration in tissue specificity observed with the 800 series small t deletion mutants did not result from a change in the enhancer region.

We showed in this study that three different SV40 mutants carrying deletions in the small t antigen gene of SV40 induce reticulum cell sarcomas in about 15% of tumor-bearing outbred Syrian hamsters injected s.c. with the virus. This result confirmed that the anomalous tumorigenic behavior is not a unique property of the dl884 mutant used in our previous studies but appears to be general for small t deletion mutants. Furthermore, our results indicated that the difference in distribution of tumor foci observed previously (4) is due to a difference in tissue specificity. The wild-type virus induced only fibrosarcomas after s.c. injection, whereas the small t deletion mutants induced lymphomas as well. As in our previous study, we observed metastatic lung nodules in some of the animals bearing s.c. fibrosarcomas induced by the small t deletion mutants. However, in the study reported here, in which many more animals were examined, we also observed a similar frequency of metastases in animals bearing wild-type-induced s.c. fibrosarcomas. Thus, the major difference in the distribution of small t deletion mutant-induced tumors within the animal appears to relate to the histologic type of tumor induced: the small t mutant induces lymphomas which are disseminated throughout the abdominal cavity and in the lung. Interestingly, lymphomas were not induced in inbred LSH hamsters injected with the dl884 mutant (data not shown); this host specificity may explain why lymphomas were not observed in earlier studies (9, 19) on tumorigenesis by small t deletion mutants.

It is possible that the anomalous tumorigenic properties of the small t deletion mutants may relate to the observation that these mutants are defective in transformation of stationary hamster cell cultures (12, 15). This finding has led to the suggestion that the small t protein may act as a tumor promoter or growth factor (11, 12). In the absence of such a factor, more time may be required for a tumor to develop to the stage at which it is detectable; thus, small t mutant-induced fibrosarcomas develop with extended latencies. The occurrence of lymphomas with relatively short latencies might also be explained on the basis of a growth factor effect. In the absence of this factor, the mutant viruses would lack the capacity to induce the proliferative state required for transformation and may preferentially transform rapidly proliferating lymphoid cells, rather than resting cells such as fibroblasts. The fact that lymphomas, leukemias, and osteogenic sarcomas are induced by the wild-type virus when it is injected by the intravenous route (2, 3) suggests that the wild-type virus is in fact able to transform lymphoid cells and osteoblasts under some conditions. However, when inoculated s.c. the wild-type virus is able to transform the slowly

FIG. 1. Typical profiles of tumor latencies. Random-bred golden Syrian hamsters (produced and housed by the Veterinary Resources Branch of the National Institutes of Health) were observed twice weekly after virus injection for evidence of tumor formation. Once observed, s.c. tumors were allowed to develop for 6 weeks or until they reached 40 to 60 mm in size, whichever came first. Abdominal tumors were allowed to develop until ascites fluid accumulation became significant or until the animal became moribund, whichever came first. All remaining hamsters were sacrificed at 18 months of age. Latency was defined as the age (in weeks) at which the hamster was sacrificed. Symbols: ●, Subcutaneous fibrosarcoma; ○, abdominal lymphoma; □, osteosarcoma.
FIG. 2. Histopathology of (A) subcutaneous fibrosarcoma and (B) abdominal reticulum cell sarcoma. Tumor samples preserved in Formalin were embedded, sliced, mounted, and stained with hematoxylin-eosin; the slides were evaluated by S. Machotka (Hazleton Laboratories) to determine the histological types of the tumors.

TABLE 1. Tumorigenesis by wild-type SV40 and small t deletion mutants

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. of hamsters injected</th>
<th>No. of hamsters with tumors</th>
<th>Frequency of abdominal lymphomas</th>
<th>Mean latency (wk) of abdominal lymphomas/mean latency of all tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt830</td>
<td>130</td>
<td>90</td>
<td>0.01</td>
<td>68/34</td>
</tr>
<tr>
<td>d884</td>
<td>106</td>
<td>51</td>
<td>0.12</td>
<td>28/62</td>
</tr>
<tr>
<td>d883</td>
<td>71</td>
<td>33</td>
<td>0.31</td>
<td>34/51</td>
</tr>
<tr>
<td>d890</td>
<td>65</td>
<td>35</td>
<td>0.17</td>
<td>40/55</td>
</tr>
</tbody>
</table>

* Number of injected random-bred Syrian hamsters reaching weaning age.

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LITERATURE CITED


