

## HUMAN STUDIES FOLLOWING ANIMAL MODELS OF TUMORIGENESIS BY ONCORNAVIRUSES<sup>1</sup>

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*Oncornaviruses, which appear to be ubiquitous in nature, have been associated with leukemias and sarcomas in many animal species. On the basis of these models, attempts have been made to determine whether overt infectious oncornavirus-like agents are associated with leukemias or solid tumors in man.*

Early studies of human leukemias and solid tumors revealed some similarities to animal malignancies induced by oncornaviruses. Particles similar to type C oncornaviruses were detected by electron microscopy in cells or plasma of patients with leukemia and in solid tumors such as Hodgkin's lymphoma, lymphosarcomas, and sarcomas. Similar particles have been detected by electron microscopy in some cell lines derived from human malignancies or by labeling the cells with radioactive uridine and measuring the release of particles with a density in sucrose gradients of 1.16 to 1.18 g/ml. Also, in findings akin to observations in placental tissues of nonhuman primates, particles similar to type C viruses were recently found in normal human placental tissue. However, the infectious nature of the particles resembling type C virions and detected in either tumor tissue or in cultured tumor cells is yet to be demonstrated.

Particles morphologically resembling the type B particles from mammary tumor virus (MTV) induced experimentally in mice have been detected in human breast cancer and in the milk of Parsi women (a population in India with a very high incidence of breast cancer) and of American women with a family history of breast

cancer. More recent studies, however, have questioned the validity of these observations. Still, while the infectious nature of the particles remains questionable, evidence suggests that they contain high molecular-weight RNA (70 S) and reverse transcriptase enzyme activity characteristic of oncornaviruses. This work has been aided by the development of a method for simultaneous detection of the enzyme activity and the high-molecular-weight RNA of known oncornavirions. Preliminary data suggest a relationship between the human particles and murine mammary tumor virus. Sera of women with breast cancer were reported to neutralize the activity of MTV, and rabbit antisera to purified MTV were reported to precipitate a soluble antigen in sera of women with breast cancer. Furthermore, the DNA synthesized *in vitro* by the enzyme of MTV (using MTV RNA as a template) was found to hybridize with polysomal RNA obtained from human mammary adenocarcinomas. No such hybridization was observed with RNA derived from other human malignancies or normal tissues, and the DNA product of the reverse transcriptases of the Rauscher strain of murine leukemia virus (R-MuLV) or of avian myeloblastosis virus also failed to hybridize with the RNA from the human mammary adenocarcinoma. It was also reported that the RNA in extracts from human mammary adenocarcinomas is a 70 S component encapsidated together with RNA-directed DNA polymerase in a particle with the

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density characteristic of oncornavirions. The DNA synthesized *in vitro* by the human 70 S RNA-enzyme complex hybridized specifically with the RNA of MTV.

Using the same procedures, the group reporting the data above also observed the presence of RNA complementary to the RNA of R-MuLV (but not to that of MTV) in other human malignancies unrelated to breast cancer. DNA obtained from R-MuLV by reverse transcription *in vitro* was found to hybridize with RNA obtained from cells of various leukemias, lymphomas (including Burkitt's lymphoma), and sarcomas. RNA in cells from various human leukemias has been reported to be a 70 S RNA complexed with reverse transcriptase. The DNA synthesized from this complex has been observed to hybridize specifically with the RNA of R-MuLV but not with the RNA of MTV or of avian myeloblastosis virus; it also appears that this DNA contains sequences common to those present in leukemic but not normal leukocytes. These results appear to be corroborated by a report on the presence of reverse transcriptase in cells of patients with acute lymphoblastic leukemia. Further work is needed to characterize the enzyme found in leukemic cells, however, since the possibility that it may be a cellular enzyme has not been ruled out.

In more recent studies it was found that cells of patients with myelogenous leukemia possess an oncornaviral-type reverse transcriptase that is distinguishable from other cell DNA polymerases and serologically related to the reverse transcriptase of primate oncornaviruses and, to a lesser extent, to that of R-MuLV. Complete oncornaviruses and primate p<sub>30</sub> antigens have been identified in cultured leukocytes of at least one patient with acute myelogenous leukemia and from a patient with transitional cell tumor.

The role of reverse transcriptase of the known animal oncornaviruses in viral carcinogenesis is not clear at present. The

findings with human malignancies tend to suggest the presence of virus-related information. They should be treated with caution, however; other workers, using DNA-DNA (other than DNA-RNA) hybridization procedures, have failed to corroborate them (although both procedures are equally sensitive in the oncornaviral model systems). Further experiments are needed with different strains of oncornaviruses to ascertain the ultimate significance of virus-related RNA and enzymes in human malignancies. The methodology now in hand is leading to a further search for specific enzymes and p<sub>30</sub> antigens of human oncornaviruses in human malignancies.

Oncornavirus C particles released by long-term cultures of myeloid cells from a patient with acute myelogenous leukemia can infect several human and animal cell strains and lines. A quantitative plaque assay has been developed for the virus based on the capacity it, like some murine leukemia viruses, has to induce syncytia in XC cells. The human virus will also rescue murine sarcoma virus pseudotypes on infection of transformed, non-virus-producing cell lines. Also, in tests for nucleic acid hybridization and in immunologic studies of viral proteins such as p<sub>12</sub>, p<sub>30</sub>, gp<sub>71</sub>, and reverse transcriptase, the human virus is shown to bear a striking resemblance to the sarcoma-associated virus in the woolly monkey. The human virus in radioimmune precipitation assays followed by gel electrophoresis is being used to screen humans for specific antibodies. Preliminary results indicate that antibodies reacting with viral proteins are widespread in the human population and that individuals develop antibodies at an early age.

Oncornavirus C particles have also been detected in human transitional cell cancers of the urinary tract. By radioimmune assay, the p<sub>30</sub> antigen of the virus (prepared from cultured cells, as well as from tissue from which the cell line was originally derived) was found to have competing

activity for the p<sub>30</sub> protein of the woolly monkey virus (SSV-1), but not for the Rauscher murine leukemia virus (R-MuLV). Extracts of normal human kidney cells and tissue were also negative. The

results suggest that these human tumors contain a cross-reacting primate type C viral antigen. A related p<sub>30</sub> protein has been found in certain other human tumors.

#### SUMMARY

Similarities have been observed for some time between oncornavirus-induced malignancies in laboratory animals and leukemias and solid tumors in man. Particles similar to type C oncornaviruses have been detected by electron microscopy both in cells or plasma from leukemia patients and in solid-tumor human malignancies such as Hodgkin's lymphoma, lymphosarcomas, and sarcomas. Likewise, particles resembling type B oncornaviruses in shape and appearance have been found in human breast cancer. In neither case has the infectious nature of the particles been confirmed. However, DNA synthesized *in vitro* by the enzyme of murine

mammary tumor virus was found to hybridize with polysomal RNA obtained from human mammary adenocarcinomas.

The presence of RNA complementary to RNA from the Rauscher strain of murine leukemia virus has been observed in other human malignancies unrelated to breast cancer. It has also been found that cells of patients with myelogenous leukemia possess an oncornaviral-type reverse transcriptase that is distinguishable from other cell DNA polymerases and serologically related to the reverse transcriptase of primate oncornaviruses.