

# INTRODUCTION TO VIRUS-CAUSED CANCERS

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Members of four different groups of animal viruses are known to cause cancer in animals. (Only two of them, the leukoviruses and herpesviruses, cause cancer in nonlaboratory situations.) All the members of these groups of viruses form integrated viral DNA in infected cells. However, the efficiencies with which they cause cancer vary by over a dozen orders of magnitude. These differences in efficiency are a result of differences in efficiency of formation and expression of the genes for neoplastic transformation. Four models of mechanisms for formation of the genes for neoplastic transformation are presented. Two involve the formation of new DNA sequences. No efficient human cancer-causing viruses are known. Therefore, it is proposed that human cancer is a result of formation of the genes for neoplastic transformation by misevolution of a normal cellular information transferring process. This misevolution is caused by chemicals, physical agents, or viruses.

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**T**HE PURPOSE OF THIS PAPER IS THREE-FOLD: 1) to describe some salient features of animal virus-caused cancers; 2) to describe why the animal virus-caused cancers are thought to provide only an analogy for the etiology of human cancer; and 3) to describe the analogy.

## ANIMAL VIRUS-CAUSED CANCERS

Many cancers in animals are known to be caused by viruses.<sup>2,21</sup> For example, Marek's disease, a lymphoproliferative disease of chickens, is caused by a herpesvirus known as Marek's disease virus. This virus is contagious among newborn chicks and has been an important cause of death of chickens in commercial flocks. Leukemia in domestic cats is caused by feline leukemia virus. It is an RNA tumor virus and is possibly contagious. Fibrosarcomas of chickens can be caused by Rous sarcoma virus. It is an RNA tumor virus and is not naturally contagious. It is only main-

tained by experimental passage. Spontaneous leukemia of Ak mice is caused by the mouse leukemia virus. It is an RNA tumor virus and is not contagious. It is passed from parental mice to progeny mice through the eggs and sperm. This type of transmission was first observed in mouse leukemia and was designated as "vertical" transmission by Gross.<sup>1</sup>

These examples are presented to illustrate that cancers with a viral etiology are found in nonprimates, that they are especially common in birds and rodents, that these virus-caused cancers are primarily leukemias and sarcomas, and that the viruses causing them differ very much from each other in the extent of their contagiousness. In spite of the fact that Marek's disease virus is contagious, most of the viruses naturally causing cancer in animals are not contagious.

A wide variety of types of tumors can be induced by viruses. For example, in a recent article, Purchase and Burmester<sup>14</sup> list the following types of tumors that are induced by avian leukosis viruses (chicken RNA tumor viruses): lymphomatosis, endothelioma, osteopetrosis, renal adenocarcinoma, hepatocytoma, and nephroblastomas including carcinoma, sarcoma, and chondroma. It is probably true that many, if not most, of the types of cancer found in man can be caused in animal by viruses.

There are many different types of animal viruses. They have been classified into groups according to whether the virus particles (virions) have A) RNA or DNA, B) have an en-

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velope or not, C) have helical or icosohedral symmetry, and D) on the size of the virus particles. Table 1 presents a list of these groups of viruses according to the way in which the viruses replicate. This list is probably not complete. New virus groups have been defined almost every year.

Some of these groups of animal viruses have no members which cause cancer. The groups of animals viruses whose members have never been shown to cause cancer include: 1) the RNA viruses with no polymerase, that is, the picornaviruses and togaviruses; 2) the viruses which contain an RNA polymerase, that is, the rhabdoviruses, orthomyxoviruses, paramyxoviruses, reoviruses, and orbiviruses; 3) the syncytium-forming viruses; 4) the arenaviruses and the coronaviruses; 5) the poxviruses, and 6) the parvoviruses. Other groups of animal viruses have members which may cause cancer in lower vertebrates. These include: 1) the leukoviruses; and 2) the papovaviruses, the adenoviruses, and the herpesviruses. (However, the papovaviruses and the adenoviruses apparently only cause cancers in experimental or laboratory situations.)

TABLE 1. Groups of Animal Viruses

RNA	
No virion polymerase	
	picornavirus
	togavirus
Virion RNA polymerase	
	orbivirus
	orthomyxovirus
	paramyxovirus
	rhabdovirus
	reovirus
Virion DNA polymerase	
	leukovirus
	syncytium-forming virus
Not characterized	
	arenavirus
	coronavirus
DNA	
Cytoplasmic replication	
	poxvirus
Nuclear replication	
	parvovirus
	papovavirus
	adenovirus
	herpesvirus

Group names have been taken from Melnick.<sup>9</sup> The RNA viruses are grouped according to the type of polymerase present in their virions. The DNA viruses are grouped according to whether the nucleic acid replicates in the nucleus or in the cytoplasm.

A common feature is found in the replication of all of the groups of viruses whose members may cause cancer.<sup>21</sup> All of the viruses which are known to cause cancer are believed to form viral DNA integrated with cellular DNA. For DNA viruses, formation of viral DNA integrated with cellular DNA is a result of recombination of the viral DNA with the cellular DNA. For RNA viruses, formation of viral DNA integrated with cellular DNA is a result of synthesis of viral DNA using the viral RNA as a template and then, apparently, recombination of this viral DNA with the cellular DNA.

From this correlation between viral DNA integrated with cellular DNA and ability to cause cancer, it is inferred that RNA viruses which do not replicate with a DNA intermediate and that RNA or DNA viruses which do not replicate in the cell nucleus will not be able to cause cancer. For example, the syncytium-forming viruses are RNA viruses which apparently form a DNA intermediate, but they do not cause cancer.<sup>13</sup> Perhaps they do not replicate their DNA in the cell nucleus so it cannot recombine with and integrate into the cellular DNA. Lack of integration with cellular DNA even though the virus DNA replicates in the nucleus may explain why parvoviruses do not cause cancer.

Poxviruses do not cause cancer. However, they do cause cell proliferation which can lead to the formation of benign tumors. The mechanism of stimulation of cell proliferation by poxviruses appears to be different from the mechanism of formation of cancers by the viruses which cause cancer in animals.

However, formation of integrated nuclear viral DNA does not in itself usually result in neoplastic transformation. For example, Haase and Varmus<sup>3</sup> have shown that the nontumorigenic RNA virus, visna, replicates through an integrated nuclear DNA intermediate, and Smith et al.<sup>16</sup> have shown that cells can be infected with a papovavirus, SV-40, and the SV-40 DNA become integrated into nuclear DNA without the cells becoming transformed into cancer cells.

Even among those virus groups whose members may cause cancer, there is a great difference in the efficiency with which the different viruses cause cancer. Table 2 lists the efficiency of tumor formation per cell generation by four members of the avian leukosis-sarcoma group of viruses (the avian RNA tumor viruses or leukoviruses). There is a large difference

in the efficiency of tumor formation by a strongly transforming RNA tumor virus (leukovirus) like Rous sarcoma virus and by a probably nontransforming RNA tumor virus (leukovirus) like Rous-associated virus-0. Both Rous sarcoma virus and Rous-associated virus-0 apparently form integrated nuclear viral DNA. The difference in efficiency of tumor formation must have another cause. I suggest that the difference in efficiency of tumor formation reflects a difference in the efficiency of formation or expression of the genes for cancer in the infected cells. The efficiency of tumor formation by DNA tumor viruses appears to be similar to or lower than that for AMV. (These efficiencies assume that immunologic factors are not operative. Immunologic factors further reduce all of these efficiencies.)

#### RELATION TO HUMAN CANCER

We must now ask whether there are any lessons for people primarily interested in human cancer in these facts gleaned from the study of animal cancer-causing viruses. I think that there are several:

1. Cancer-causing viruses exist in animals, especially in chickens and mice. These viruses can cause a wide variety of cancers in these species.
2. These animal cancer-causing viruses are easily recovered from or demonstrated in the cancers they cause. Even in cases where the infectious virus is not present in the tumors, it can be recovered by fusion with permissive cells or demonstrated by immunologic methods or by nucleic acid hybridization.
3. Cancer is usually a very rare response to the replication of these cancer-causing viruses. Strongly transforming viruses like the Rous sarcoma virus are very rare. They are maintained only by experimental passage in the laboratory. The wild or natural animal cancer-causing viruses, that is, viruses which are maintained without laboratory transmission, usually cause tumors with very low efficiencies and often only in species which are not natural to the viruses.
4. No efficient or strongly transforming human cancer-causing virus is known. As discussed above, such viruses, when they are present in cancers, are easy to isolate

TABLE 2. Efficiency of Tumor Formation by Different Avian Leukosis-Sarcoma Viruses

Virus	Efficiency per cell generation
RSV	1/10
AMV	1/10 <sup>5</sup>
LLV	1/10 <sup>12</sup>
RAV-0	1/10 <sup>15</sup>

RSV—Rous sarcoma virus; AMV—avian myeloblastosis virus; LLV—lymphoid leukosis virus; RAV-0—Rous-associated virus-0.

Immunologic factors are assumed to be inoperative. The efficiencies are guesses based upon the latent period for tumor formation in sensitive chickens. Only the efficiency for RSV is known with precision. The efficiency for AMV may be much lower.

or to demonstrate. However, such strongly transforming viruses are very rare even in animals. In humans, there is only some suggestive indirect evidence that viruses may be in some way related to some cancers. However, this relationship does not indicate the natural existence of a strongly transforming human cancer-causing virus.

5. Animal cancer-causing viruses do not indicate the etiology of human cancer. However, they may be relevant to an understanding of the etiology of human cancer by providing analogies for the formation and expression of the genes for neoplastic transformation in human cancer.

#### MECHANISMS OF NEOPLASTIC TRANSFORMATION

Figure 1 indicates some of the ways in which animal cancer-causing viruses cause the formation of genes for neoplastic transformation in cells with integrated viral DNA genomes.<sup>17,19,20</sup> I shall then suggest that in human cancers the genes for neoplastic transformation are formed in a way analogous to one of these.

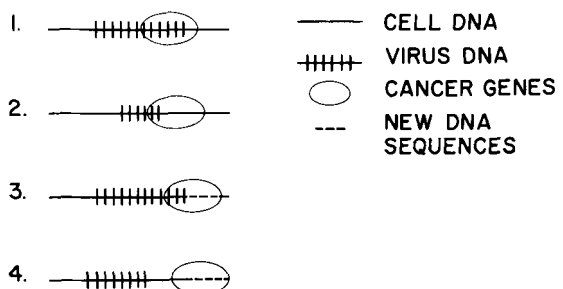


FIG. 1. Possible mechanisms for formation of cancer genes in virus-caused cancers.

In Model 1, the virus genome contains genes for neoplastic transformation and, therefore, the cellular DNA containing integrated viral DNA contains these genes. This is the mechanism of cancer formation by Rous sarcoma and murine sarcoma viruses. The existence of viral mutants which are temperature-sensitive for the cancer phenotype supports this mechanism.

In Model 2, the genes for neoplastic transformation are formed as a result of integration of the viral DNA with cellular DNA; for example, the viral DNA integrates in the center of a cistron and inactivates it, or as a result of interaction of the integrated viral DNA and the adjoining host DNA; for example, a viral promoter turns on transcription of adjoining sequences. (This interaction could be on both sides of the viral genome rather than just one as indicated.) This mechanism would lead to cancer only when the virus integrates at a special site in the cellular DNA. Such a mechanism may be operative for polyoma virus. Polyoma virus has a low efficiency of transformation, but a mutant polyoma virus exists which is temperature-sensitive for some, but not all, of the neoplastic properties of the infected cell.

In Model 3, the cancer genes are formed by the interaction of the integrated viral DNA and the contiguous cellular DNA. However, this cellular DNA contains new DNA sequences. The difference between Models 2 and 3 involves the presence of the new DNA sequences. The new sequences could be formed at the time of integration of the viral DNA

or during subsequent replication of this integrated viral DNA.

In Model 4, the genes for neoplastic transformation are neither in the virus DNA nor in DNA which is next to the virus DNA. The genes for neoplastic transformation are in another place in the cell genome and, again as in Model 3, involve new DNA sequences. These new sequences are formed after integration of the viral DNA as a result of the action of viral products on the host DNA. (A popular recent hypothesis, the oncogene hypothesis, is somewhat like Model 4. But in the oncogene hypothesis the genes for neoplastic transformation are present in all normal cells.<sup>4</sup> Since there has been no evidence in support of this hypothesis, and since there are strong theoretical arguments against it, for example, the lack of selective pressure to maintain these genes in all cells,<sup>19</sup> I have not illustrated it.

#### NEW DNA SEQUENCES

Some of these models require the formation of new DNA sequences. There is now a fair amount of evidence that indicates that new DNA sequences exist in RNA tumor viruses. These experiments involve both genetic experiments and nucleic acid hybridization.

Wyke<sup>22</sup> showed that two different mutants of the B77 strain of avian sarcoma virus temperature-sensitive for neoplastic transformation could complement each other (Fig. 2). That means that if two different mutants were present in the same cell at the nonpermissive temperature, the cell was transformed. However, he found that the B77 virus mutants were not complemented by mutants of either Prague strain or Schmidt-Ruppin strain of Rous sarcoma virus which were temperature-sensitive for neoplastic transformation (Fig. 2). This result indicates that the genes for neoplastic transformation in the B77 strain of avian sarcoma virus and in the two strains of Rous sarcoma virus are different in that their products cannot complement each other.<sup>8</sup> This failure of complementation might indicate that the genes for neoplastic transformation are different either in their function or in their detailed molecular architecture. This difference must have occurred either at the time of the origin of the genes for neoplastic transformation or in their later evolution. Therefore, new DNA sequences must have appeared either when these viruses originated or in their later replication.

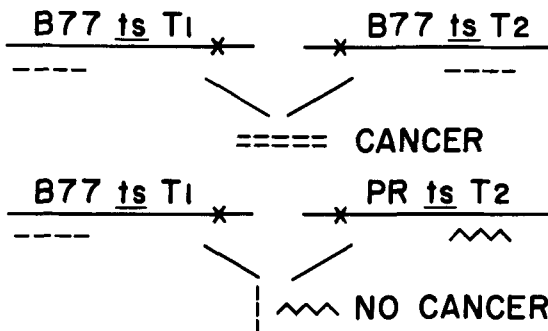


FIG. 2. Diagram of virus complementation experiment. Chicken embryo fibroblasts were infected with two different strains of B77 virus temperature-sensitive for neoplastic transformation (top), or with one of these B77 viruses and one strain of Prague Rous sarcoma virus temperature-sensitive for neoplastic transformation (bottom). The presence or absence of morphological transformation at the nonpermissive temperature, 41C, was measured. (Wyke, 1973).<sup>22</sup>

Neiman<sup>11,12</sup> studied the hybridization of labelled RNAs of Rous sarcoma virus and Rous-associated virus-0 with DNA from uninfected chicken cells and chicken cells infected with Rous sarcoma virus (Fig. 3). He found that there were only about 45% of the nucleic acid sequences of Rous sarcoma virus in the DNA of uninfected chicken cells, and nearly 100% of the nucleic acid sequences of Rous sarcoma virus in the DNA of Rous sarcoma virus-infected cells. He also found there were nearly 100% of the nucleic acid sequences of Rous-associated virus-0 in the DNA of uninfected chicken cells. Since the genomes of Rous-associated virus-0 and Rous sarcoma virus are apparently of the same size, the Rous sarcoma virus apparently has in its genome a large portion of nucleic acid sequences which are not present in uninfected chicken cell DNA. Since Rous sarcoma virus originated in a chicken, these new sequences must have appeared at the time of origin of the virus or in later passages.

Scolnick et al.<sup>15</sup> compared by nucleic acid hybridization the sequences of the RNA of Kirsten murine sarcoma virus with the sequences of the RNA of Kirsten murine leukemia virus and rat leukemia virus. They showed that the Kirsten murine sarcoma virus had nucleic acid sequences which were present in the Kirsten murine leukemia virus as well as nucleic acid sequences which were present in the rat leukemia virus. These results indicate that there was recombination between the Kirsten murine leukemia virus and rat leukemia virus. However, this recombination not only led to the formation of a recombinant virus, but it led to new properties in this recombinant virus, that is, the ability to cause neoplastic transformation. Since neither of the parents had this property, new DNA sequences probably appeared.

Kang<sup>7</sup> has shown that the RNA of reticuloendotheliosis viruses, a group of avian RNA viruses classified separately from the avian leukosis-sarcoma viruses, shares few or no nucleic acid sequences with DNA of uninfected chickens, quails, Muscovy ducks, turkeys, and pheasants. Reticuloendotheliosis viruses replicate through a DNA intermediate, and their DNA polymerases have serologic relationships to avian cellular DNA polymerases.<sup>10</sup> These serologic relationships might indicate that these viruses originated at some time from cellular information. However, they

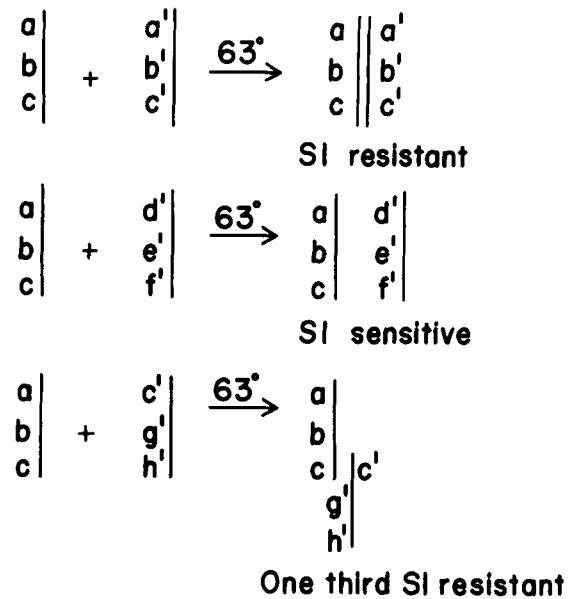


FIG. 3. Nucleic acid hybridization test for differences in nucleic acid sequences. abc is complementary to a'b'c'. abc is not complementary to d'e'f'. SI is a nuclease specific for single-stranded nucleic acids.

now apparently have nucleic acid sequences different from those in cellular DNA.

These results indicate that new nucleic acid sequences are created and can be found in RNA tumor viruses.

We then shall ask if there are possible mechanisms for the origin of new nucleic acid sequences.

All of the cases I have discussed involve RNA viruses which replicate through a DNA intermediate. In addition, we have demonstrated that uninfected chicken cells have endogenous RNA-directed DNA polymerase activity.<sup>5,6</sup> The presence of this activity indicates that some information in normal cells is transferred DNA to RNA to DNA, as predicted by the protovirus hypothesis<sup>18</sup> This kind of information transfer might be involved in the generation of new nucleic acid sequences. These new sequences could originate at the junctions of recombinates. For example, if one sequence was *abcdefghijkl* and another sequence was *lmnopqrst*, and each sequence was transcribed into RNA and then into DNA, and there was recombination of this DNA and the original DNA so that *nopq* was inserted between *a* and *b*, the new sequences *an* and *qb* would be formed. In addition, there could be mutations in this DNA to RNA to DNA information transfer. Some

experiments with RNA tumor viruses suggest that the frequency of mutations in this kind of transfer may be very high.

However, the rates of these two processes are probably not sufficient to explain the

#### NORMAL CELL

————— CELL DNA

#### CANCER CELL

—————  NEW DNA SEQUENCES  
CANCER GENES

FIG. 4. Comparison of genomes of normal cells and "spontaneously-induced" cancer cells.

origin of all of the new nucleic acid sequences which we have discussed. Therefore, I propose that there exists in DNA to RNA to DNA information transfer a process which can generate new nucleic acid sequences. These new sequences are probably not random, but depend on the nature of the "templates" and the polymerases used. The new nucleic acid sequences would normally be generated for some normal cellular functions, such as development of antibody diversity or of memory. But, in addition, this generation could be the process which occasionally leads to the origin of new sequences for RNA tumor viruses and the new sequences for neoplastic transformation in the absence of viruses (Fig. 4).

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