RNA-DEPENDENT DNA-POLYMERASE ACTIVITY IN HUMAN TUMORS

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Summary

RNA-dependent DNA-polymerase activity is present in specimens of freshly excised human medulloblastoma. This activity is demonstrated in the cell homogenates and activity remains in the supernatant fluid at 30,000g after 30 minutes and pellets at 100,000g in 2 hrs. Detergent is necessary to elicit significant activity. The enzyme activity was determined using a poly(A)·oligo(dT) template. Retinoblastoma, neuroblastoma and ocular malignant melanoma also showed activity but in much lower amounts than medulloblastoma. Normal human orbital connective tissue, cerebellum and retina, similarly studied, failed to show activity with this template. Thus, by these methods a RNA-dependent DNA-polymerase activity is demonstrated in human tumors, but not in the corresponding normal tissues.

Introduction

The finding by Temin, Mizutani (1) and Baltimore (2) of a specific RNA-dependent DNA-polymerase related to RNA oncogenic viruses has opened new pathways into the investigation of the possible viral etiology of human cancer. Using the synthetic template poly(A)·oligo(dT), Ross et al. (3) showed that it was possible to distinguish between an enzyme with reverse-transcriptase-like properties found in normal cells, and the true viral enzyme. This finding was recently confirmed by Goodman and Spiegelman (4).

In recent years increasing evidence has accumulated which suggests that viruses play a role in the cause of many types of human cancer. Evidence has consisted primarily in the demonstration of 1.) virus-like particles in tumor cells (5-9); 2.) the demonstration of common tumor-specific antigen and antibodies against particular forms of human cancer (10); 3.) analogy to spontaneous and experimental animal malignancies (11). Certain epidemiologic, clinical and histopathologic features of retinoblastoma, an intraocular tumor affecting infants and young children, are suggestive of a viral etiology (12).

Medulloblastoma and neuroblastoma are tumors occurring elsewhere in the body, primarily affecting patients in the same age group as retinoblastoma and having a similar histology and course. Malignant melanoma is the principal primary intraocular tumor of adults. Implication of a viral origin for malignant melanomas is based upon the demonstration of tumor specific antigens in the cutaneous form of this tumor (10). The present experiments were carried out to determine if RNA-dependent DNA-polymerase is present in these human tumors and the corresponding normal tissues.

Materials and Methods

Tumor and control tissues used in this study were obtained at the time of surgery or autopsy and stored at -20°C. Identification, pathologic diagnosis and specific comments regarding the individual specimens are listed in Table 1.

TABLE 1: TISSUES USED

Pathologic Diagnosis and Patient Identification	Comment
Medulloblastoma (M.M. E71-324)	Anaplastic, highly cellular tumor
Retinoblastoma (B.R. Y23)	Bilateral involvement: no known familial involvement
Choroidal Malignant tumor (S.B. E71-210)	Spindle-B type; no extraocular involvement
Connective Tissue (E.V. E71-306)	From orbital biopsy; Normal appearing fibroblasts subsequently cultured.
Normal Cerebellum (B.M. Y-70)	Infant with suspected medulloblastoma; biopsy taken; no tumor found
Normal Cerebellum (J.C. Y-72)	Cirrhosis secondary to infectious hepatitis eighteen years previously; post-op. Portacaval shunt
Normal retina and choroid (J.U. E71-654)	Autopsy specimen; normal eyes
Neuroblastoma (C.M. Y64)	Removed from abdomen; extensive spread; site of primary uncertain

Polyribonucleotides were a product of Miles Laboratories, Elkhart, Indiana; concentrations were determined from extinction coefficients provided by the manufacturer and an assumed MW of 100,000. Oligodeoxyribonucleotides were obtained from Collaborative Research, Waltham, Mass. Chain lengths were listed

at 12-18 units and their concentrations were determined from the extinction coefficient of the respective deoxyribonucleotides. The tritium labelled thymidine triphosphate was obtained from New England Nuclear Corp. (18.2 Curies/mmole). The unlabelled triphosphates were obtained from P-L Biochemicals.

Avian myeloblastosis virus was kindly provided by Drs. J. Chirigos and J. Beard, as a plasma suspension. The virus was further purified by differential centrifugation and concentrated with 10% polyethylene glycol (Matheson Coleman Bell PEG-6000) in the presence of 0.5 M NaCl. The polymerase from this virus was used to establish assay methods and purification techniques.

Each reaction mixture was incubated for 60 min. at 35° and contained, in the complete system (unless stated otherwise), in 0.1 ml: 0.02M Tris (pH 8.0); 0.3mM manganese chloride; 60mM sodium chloride; 30mM dithiothreitol; 0.8% Nonidet P-40; 0.8μM ^{3}H-thymidine triphosphate (14.5 x 10^{3} cpm/pmole); 0.8mM ATP. Where indicated, the polymer concentrations were: poly(A) 1.3 x 10^{-5}M; poly(dT) 2 x 10^{-6}M; oligo(dT) 2.1 x 10^{-6}M. Unless stated otherwise, 10μl of 0.6 mg protein per ml of tissue homogenate was used.

Results

Two different templates (synthetic duplexes) were used in assaying for DNA polymerase activity. These were poly(A)·oligo(dT) [oligo(dT) is 12-18 units in length] and poly(A)·poly(dT). Enzyme activity toward the former template is referred to as DNA polymerase(oligo) and the latter activity as DNA polymerase (poly).

As seen in Table 2, medulloblastoma shows both DNA polymerase(oligo) and DNA polymerase(poly) activity. Whereas fibroblasts show only DNA polymerase (poly) activity. Control specimens of cerebellum show no activity towards either template. One of the specimens of cerebellum used was from exploratory surgery on a patient who was clinically thought to have medulloblastoma, and the other from a patient with liver disease. Both were histopathologically normal.

TABLE 2: DNA POLYMERASE ACTIVITY WITH VARIOUS TEMPLATES

Tissue	Mg protein per ml	Template	pmol [^{3}H] TMP incorporated x 10
Medulloblastoma	0.95	———	<.6
		poly(A) oligo(dT)	620.
		poly(A) poly(dT)	310.
		poly(A)	<.6
		oligo(dT)	<.6
		poly(dT)	<.6
		Calf Thymus DNA	<.6
Fibroblasts	0.28	———	3.2
		poly(A) oligo(dT)	3.2
		poly(A) poly(dT)	20.8
		poly(A)	.6
		oligo(dT)	3.2
		poly(dT)	3.2
		Calf Thymus DNA	3.2
Normal Cerebellum	0.61	———	<.6
		poly(A) oligo(dT)	<.6
		poly(A) poly(dT)	<.6
		oligo(dT)	<.6
		poly(dT)	<.6

At the present time we are unable to tell whether the DNA polymerase(oligo) and DNA polymerase(poly) activities associated with meduloblastoma are from the same enzyme. However, the fact that the two activities are found in different relative amounts depending upon the treatment of the homogenate (e.g., polyethylene glycol precipitation of the enzyme results in lowering the poly/oligo activity ratio), leads us to believe that the activities represent separate enzymes.

The effects of various metal ion concentrations on the medulloblastoma enzyme are shown in Table 3, and the time course of the reaction can be seen in Figure 1.

TABLE 3: DNA POLYMERASE METAL ION DEPENDANCE

Tissue	Salt	Metal Ion	pmol [^{3}H] TMP incorporated x 10
Medulloblastoma	60mM KCl	———	<.6
	"	6mM Mg	1.8
	"	0.006mM Mn.	<.6
	"	0.30mM Mn	31.5
	"	0.45mM Mn	24.1
	"	0.60mM Mn	17.4
	60mM Na Cl	0.3mM Mn	46.5

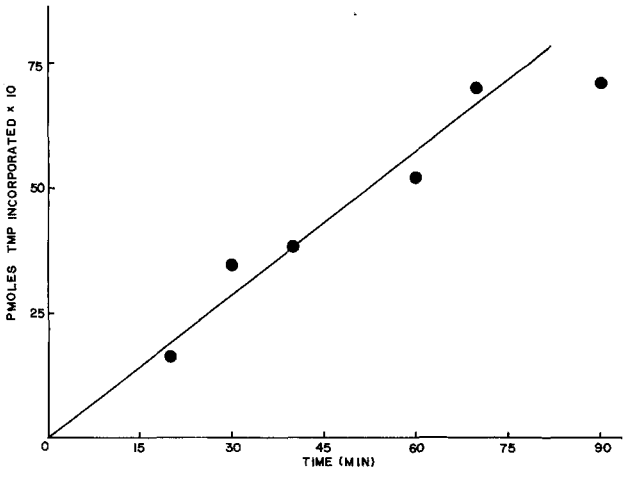

Figure 1: Poly(A)·oligo(dT) as a template for the DNA polymerase of medulloblastoma

It has been previously shown that detergent is needed to obtain polymerases from animal viruses. The detergent requirement of the enzyme in the tumors in the present investigation were studied by differential centrifugation (see Table 4). When the tissue homogenate is subjected to 30,000g centrifugation it is found that approximately 88% of the activity in both the supernatent fraction and the pellet requires detergent treatment inorder to be evident. If the 30,000g supernatent solution is further spun at 100,000g, only 17% of the

TABLE 4: CENTRIFUGATION STUDY AND DETERGENT REQUIREMENT

Treatment	pmol [^{3}H] TMP incorporated x 10	% Activity Requiring Detergent	pmol [^{3}H] TMP inc. mg protein/ml
30,000g pellet	600	90%	8.0
30,000g supernate	143	88%	—
100,000g supernate	123	17%	35.8
100,000g pellet	168	92%	140.0

activity in the supernatant fraction requires detergent, while 91% of the enzyme in the pellet shows a detergent requirement. It is also found that the specific activity of the enzyme increases during this procedure.

The data shown in Table 5 shows the possible presence of RNA-dependent DNA polymerase(oligo) in retinoblastoma, neuroblastoma and choroidal malignant melanoma. No activity was found in the corresponding normal tissues.

TABLE 5: DNA POLYMERASE ACTIVITY IN OTHER TISSUES

Tissue	Mg protein per ml	Template	pmol [^{3}H] TMP incorporated x 10
Retinoblastoma	0.25		<.6
		poly(A) oligo(dT)	6.2
(no detergent)		poly(A) oligo(dT)	<.6
		oligo(dT)	<.6
		poly(A)	<.6
Neuroblastoma	16.0		1.8
		poly(A) oligo(dT)	20.1
		oligo(dT)	1.9
Melanoma	1.0		<.6
		poly(A) oligo(dT)	7.6
(no detergent)		poly(A) oligo(dT)	<.6
		poly(A)	<.6
Normal Retina	0.4	poly(A) oligo(dT)	<.6
Normal Choroid	0.4	poly(A) oligo(dT)	<.6

Discussion

The above results indicate a RNA-dependent DNA polymerase activity in human medulloblastoma and apparent presence in retinoblastoma, neuroblastoma and choroidal malignant melanoma, that is not demonstrated in the corresponding normal tissues. This activity is similar to reverse transcriptase related to oncogenic RNA viruses (1, 2).

It is found that caution is necessary in the interpretation of findings based solely on the use of synthetic templates, for it has been known for sometime (13) that the DNA polymerase of *Escherichia coli* accepts poly(A)·poly(U) as a template to synthesize poly(dA)·poly(dT). Indeed, it was later reported by Todaro and colleagues (14) that human fibroblasts show reverse transcriptase type activity towards poly(A)·poly(dT). Subsequently, however, Ross *et al*. (3) found that it was possible to distinguish between a normal enzyme in mouse cells, which has some of the properties of reverse transcriptase (i.e. it can use poly(A)·poly(dT) as a template) and the viral enzyme which can use a poly(A)·oligo(dT) as a template. Ross *et al*. (3) also separated these two enzymic activities from cells infected with an RNA virus and showed it was possible to distinguish between the two enzymes. Similar observations were obtained by Goodman and Spiegelman (4) who differentiated between reverse transcriptase of

an RNA animal tumor virus and other known DNA polymerases. They showed that certain oligomerhomopolymer complexes serve as excellent distinguishing agents. Further work on the enzyme template characteristics have been carried out by Baltimore and Smoler (15).

The present data based on the techniques described by the above workers represents the finding of similar activity towards poly(A)·oligo(dT) in a human tumor. This activity has previously been found to be associated with animal RNA viruses, and in virus-like particles isolated from the milk of patients with breast cancer (16). The present results may be interpreted as showing either a true viral reverse transcriptase (indicating a possible relationship to a RNA-oncogenic virus) or a new enzyme with properties different from those of any known DNA polymerase. The finding that activity can be pelleted at 100,000g indicates that the enzyme is associated with a larger particle. This could result from attachment to cell membranes, polyribosomes, or inclusion within a viral particle. The observation that either freezing and thawing, or detergent treatment, is necessary to release activity, is consistent with an enzyme located within a viral particle. Further experiments are being carried out in order to differentiate between the above possibilities.

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