Pattern of Isoaccepting Transfer RNAs Common to 26 Patients with Hodgkin’s Disease

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ABSTRACT

The elution profiles of aminoacyl transfer RNAs from Hodgkin’s tumors have been compared with the corresponding patterns from normal splenic tissue. Aminohezyl-Sepharose and reversed-phase 5 chromatography have been used in the fractionation studies. Three peaks of acceptor activity have been observed in phenylalanyl, histidyl, aspartyl, and asparaginyl transfer RNAs. A second peak was shown in the case of tyrosyl and methionyl transfer RNAs. Seryl transfer RNA showed no change in elution profile; namely, a single species was observed in both normal and tumor transfer RNAs. These observations are confirmation that Hodgkin’s disease is a malignant disease. The uniformity of the extra species of tRNA suggests that there is a commonly occurring aberration in the cell of origin of the Hodgkin’s tumor.

INTRODUCTION

Hodgkin’s disease has been known to exist as a clinical entity since 1832. During that time, it has become a model for both clinical and basic research. On the clinical side, it has been possible to study a very distinctive pathology which features the Reed-Sternberg cell, considered to be pathognomonic of the disease. Early in the 20th century, this cell was thought to be a variety of a giant cell seen in tuberculosis. The clinical features of the disease, i.e., lymphadenopathy, occasionally splenomegaly, and the constitutional symptoms of night sweats, fever, and weight loss, were all believed to fit this hypothesis. It is only since 1940 that evidence has accumulated that this is indeed a malignant process. The institution of linear megavoltage radiotherapy and combination chemotherapy has virtually made “cure” a reality for a high percentage of patients. What has never been explained is the true nature of the pathogenesis of the disease.

With the observation of Borek et al. (1, 2) in the 1970’s that methyltransferases are aberrantly hyperactive in every malignant tumor and that products of the action of these enzymes may be excreted in the urine, we used this approach to seek a marker of disease activity in patients with Hodgkin’s disease. It is only since 1940 that evidence has accumulated that this is indeed a malignant process. The institution of linear megavoltage radiotherapy and combination chemotherapy has virtually made “cure” a reality for a high percentage of patients. What has never been explained is the true nature of the pathogenesis of the disease.

MATERIALS AND METHODS

Spleens were obtained from 26 patients who underwent elective laparotomy for Hodgkin’s disease. They were immediately transported to the laboratory on ice and then weighed and sliced into thin sections. Tumor nodules were separated as completely as possible from normal adjacent spleen tissue. In cases where the spleen was completely involved, tissues from other macroscopically normal spleens were used as controls. Otherwise, normal splenic tissue from individual patients were used as a source of control materials. Nineteen patients were studied by AH-Sepharose chromatography, and 15 patients were studied by RPC-5 chromatography. This included a group of 8 patients who were studied by both methods. Cochromatography of normal and tumor tRNA was performed in instances when they were isolated from the same spleen.

AH-Sepharose was obtained from Pharmacia (Uppsala, Sweden), and RPC-5 packing (polychlorotrifluoroethylene (Plaskon CTFE) coated with Adogen 464) was kindly donated by Dr. Sylvia Kerr (University of Colorado Medical Centre, Denver, Colo.). The 3H-labeled amino acids were purchased from the Radiochemical Centre, Amersham, England, and had the following specific activities (Ci/mmole): L-[methyl-3H]methionine, 12.0; L-[3-3H]serine, 19.0; L-[3,5-3H]tyrosine, 45.0; L-[4-3H]phenylalanine, 29.0; L-[2,3-3H]aspartic acid, 15.0; L-[2,5-3H]histidine, 42.0. L-[2,3-3H]asparagine (13.9 Ci/mmole) and Aquasol were purchased from New England Nuclear, Boston, Mass.

Preparation of tRNA. tRNA was prepared from normal and control tissues by a modified method of Yang and Novelli (22) as described by Friedman (5). The precipitate was loaded on a column of DEAE-cellulose that was previously equilibrated with 0.1 M Tris-HCl buffer (pH 7.5). tRNA was eluted with 1 M NaCl containing 0.1 M Tris-HCl (pH 7.5) and precipitated by adding 0.1 volume of 20% potassium acetate (pH 4.5) and 2.5 volumes of cold ethanol. It was collected by centrifugation and then dissolved in 0.1 M Tris-HCl.

Preparation of Aminoacyl-tRNA Synthetases. Synthetases were prepared according to a slightly modified version of the method of Pearson (18). Splicic tissue was homogenized with 3 to 4 volumes of buffer [0.05 M Tris-HCl (pH 7.5), 0.01 M MgCl2, 0.01 M β-mercaptoethanol, 0.1 M KCl, 1 mM EDTA, 0.25 M sucrose, and 15% glycerol]. The homogenate was centrifuged for 10 min at 10,000 rpm in a Beckman J-21B centrifuge to remove solid debris and lipids. The aqueous solution was withdrawn by aspiration from beneath the lipid layer and clarified by ultracentrifugation for 1 hr at 30,000 rpm (105,000 x g) in a Beckman L5-65 centrifuge. An aliquot of the supernatant

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was diluted with Buffer A [0.01 M potassium phosphate (pH 7.5), 0.02 M β-mercaptoethanol, 1 mM EDTA, 4 mM MgCl₂, and 15% glycerol] and then layered on a Sephadex G-100 column equilibrated with Buffer A plus 5 mM KCl. The active fractions were directly applied to a DEAE-cellulose column equilibrated with the same buffer used in the Sephadex column, except that β-mercaptoethanol was replaced with 2 mM reduced glutathione. The aminoacyl-tRNA synthetases were eluted with the same buffer containing 0.3 M KCl. Aliquots (5 ml) were placed in small vials and promptly frozen and stored in liquid nitrogen at −196°.

**Chromatography on AH-Sepharose 4B Columns.** The amino acids studied for acceptance by normal and tumor tRNAs by a modification of previously described methods (4, 8) were phenylalanine, serine, tyrosine, histidine, methionine, and aspartic acid. The column (0.9 x 60 cm) was equilibrated with 50 ml of 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M MgCl₂, 0.006 M β-mercaptoethanol, and 0.001 M EDTA. Between 1 and 5 A₂₆₀ units of purified tRNA were dissolved in 1 ml of the buffer (as above) and placed on the column. A reverse gradient of ammonium sulfate (1.3 M to 0) was used for the elution, the flow rate being 40 ml/hr. Between 40 and 50 fractions (10 ml each) were collected and analyzed for amino acid acceptance. Two volumes of water were added to dilute the high concentration of salt in the first few fractions.

The reaction mixture contained 1 ml of the tRNA fraction, 50 μmol of Tris-HCl (pH 7.5), 50 μmol of KCl, 50 μmol of MgCl₂, 4 μmol of ATP, 20 nmol of ³H-labeled amino acid, and 0.05 ml of (approximately 20 μg of protein) of aminoacyl-tRNA synthetase in a total volume of 2 ml.

The mixture was incubated at 37° for 30 min. An equal volume of ice-cold 10% trichloroacetic acid was then added, and the tubes were left standing on ice for 30 min and then filtered through Whatman GF/C glass fiber filters. After washings first with 50% (v/v) ethanol-ether and then with ether, the filters were air dried and counted with 10 ml of a toluene-based scintillation fluid. A blank containing no tRNA was also processed.

**Preparation of ³H- and ¹⁴C-labeled Aminoacyl-tRNA.** ³H-labeled aminoacyl-tRNA from tumors and ¹⁴C-labeled aminoacyl-tRNA from normal spleen tissue were prepared by incubation of 0.1 to 0.2 mg tRNA for 30 min at 37° with aminoacyl-tRNA synthetase (0.2 mg protein) in a 1-ml reaction mixture containing 0.1 M Tris-HCl (pH 7.5), 10 mM KCl, 10 mM MgCl₂, 2 mM ATP, and 1 μCi ¹⁴C-labeled amino acid or 10 μCi ³H-labeled amino acid. Then 0.1 ml of 1 M sodium acetate buffer (pH 4.0) and an equal volume (1 ml) of 88% phenol were added to the reaction mixture, shaken at room temperature for 10 min and then centrifuged for 10 min at 10,000 rpm. The labeled aminoacyl-tRNA was precipitated from the aqueous layer by the addition of 2.5 volumes of cold ethanol and kept at −20°.

**RPC-5 Column Chromatography.** A 0.9- x 30-cm RPC-5 column was equilibrated with 0.01 M sodium acetate buffer (pH 4.7) containing 10 mM MgCl₂, 2 mM β-mercaptoethanol, and 0.4 M NaCl (Buffer B). Aminoacyl-tRNAs from tumors and normal spleen tissues (labeled with ³H- and ¹⁴C-labeled amino acids, respectively) were suspended in 2 ml of Buffer B and placed on an RPC-5 column. The column was then washed with 10 ml of Buffer B and eluted with a linear gradient of NaCl (50 ml each of 0.5 M and 0.9 M NaCl) in 0.01 M sodium acetate buffer (pH 4.7) containing 0.01 M MgCl₂ and 2 mM β-mercaptoethanol. Fractions of 1.3 ml were collected at a flow rate of 1 ml/min. Ten ml of Aquasol were added to each and then counted in a liquid scintillation counter.

**RESULTS**

The typical elution profiles of Phe-tRNA, Ser-tRNA, Tyr-tRNA, Met-tRNA, His-tRNA, and Asp-tRNA from normal and...
tumor tissues obtained from the spleen of a patient with Hodgkin's disease, as studied by AH-Sepharose chromatography, are shown in Chart 1.

Chart 2 shows results of cochromatographic studies performed on a RPC-5 column. tRNAs were obtained from normal and tumor tissues from a Hodgkin's patient's spleen, and the study included Asn-tRNAs besides those listed above. No significant variations were observed when comparing the elution profiles of individually run samples with those run in pairs.

The tRNAs studied were observed to have at least one extra species in each of the tumors, except Ser-tRNA which showed no significant differences between normal and tumor tissue. All others had significant modifications, the tumor tRNA being resolved into 2 or more species in each case.

In the case of Phe-tRNA, 3 distinct peaks have been observed. Tyr- and Met-tRNAs were observed to have 2 peaks. His- and Asp-tRNAs also had a third tumor-specific peak, the resolution being improved on the RPC-5 columns.

We have compared our results, both qualitatively and quantitatively, between each subtype of Hodgkin's disease as classified by Lukes and Butler (12). Since 17 of the 26 patients studied were of the nodular sclerosing group, we subdivided these into low-, intermediate-, and high-grade disease and have correlated these with the lymphocyte predominance, mixed-cell, and lymphocyte depletion subtypes, respectively. Since the presence or absence of constitutional symptoms would not be expected to have an effect on the tumor tRNAs studied, this factor was not taken into consideration. The relative distributions of counts in the Phe- and His-tRNAs of some patients with matched histology are shown in Table 1.

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<th>Patient subtype</th>
<th>Phenylalanine</th>
<th>Histidine</th>
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Table 1: Distribution of counts in phenylalanyl and histidyl isoacceptor tRNA peaks in the different subtypes of Hodgkin's disease.
T. L. Murphy and I. A. Cooper

DISCUSSION

In a previously reported study, we observed an elevation in methyltransferase activity in Hodgkin’s tumors when compared with normal spleen (14). A number of authors have reported similar changes in every malignant solid tumor examined, whereas benign tumors do not have elevated levels of enzyme activity (20). We have also observed an increase in the urinary excretion of modified bases of tRNA such as pseudouridine and methylated guanosines (3, 19). Our studies on the bulk tRNA extracted from tumors have shown some evidence for an increase in the levels of some nucleosides, but this has been inconclusive (14). This could most probably be explained by the fact that there are usually only a few altered species among the total pool of tRNAs. The other problem relates to the fact that Hodgkin’s tumors contain not only the “atypical histiocyte” (thought to be the malignant Hodgkin’s cell) but also a variety of other cells, such as plasma cells or eosinophils. Hodgkin’s disease is also characterized by 4 histologically recognizable subtypes, with varying numbers of tumor cells. This fact adds to the difficulty of interpretation of the significance of the observations made on urine of patients and methyltransferase activity in the tumors. In view of this, it therefore seemed essential that we study the individual isoaccepting species.

Comparable elution profiles were obtained on AH-Sepharose and RPC-5 columns, although a better definition of peaks was generally observed with RPC-5 column chromatography. This improvement was most significant in the separation of isoaccepting Met- and Asp-tRNAs. In 2 cases, it was difficult to resolve the tumor-specific peaks from the major peak when run on AH-Sepharose columns. The broader based major peak was thought to consist of unresolved isoacceptors. When rechromatographed on RPC-5 columns, however, a successful separation was achieved. This might be due to the fact that the presence of certain bases contributes to the absorption of tRNAs on RPC-5. This support is therefore sensitive to small differences in base composition.

The additional phenylalanyl species observed in our study of tumor tRNAs are in keeping with similar observations which have been made by other workers (6, 11, 13). Kuchino and Borek (11) have found a specific modification in the Phe-tRNA of a Novikoff hepatoma, which is a homogeneous tumor, and have also made similar observations in the Ehrlich ascites cell line. The Phe-tRNA has also been shown to contain 2 supernumerary methylated bases, one of which is 1-methylguanine. The significance of this change is unsure, but it would appear to be a new occurrence in the malignant cell and not an attribute of the cells of origin.

The additional peaks that we have observed in the Tyr-tRNA, His-tRNA, Asp-tRNA, and Asn-tRNA are also of particular interest since these tRNAs belong to a class that recognizes XA\(^{\text{V}}\) codons (X represents A, U, G, and C). It has been shown that tRNAs in this class contain the modified nucleoside Q (queueine) and hexose-containing Q derivatives in the first wobbling position of the anticodon (7, 10, 16, 17).

Since it is not possible to present the results obtained quantitatively from all the patients studied, we have selected Phe- and His-tRNAs of some patients from the different subtypes of Hodgkin’s disease in order to observe any possible correlation that might exist between them. These aminoacyl-tRNAs were chosen because of their relationship to the 2 hypermodified bases, namely, the O\(_2\)-Wye (formerly called “Y”) and Q bases. All the samples appear to have a fairly uniform pattern of distribution of counts. It would appear that the histological subtype does not influence the pattern of isoacceptors.

It is generally believed that the isoacceptor tRNA species in tumor tissues are undermodified tRNAs which have primary structures that are the same as or similar to those of tRNAs present in normal tissues. At present, it is unclear as to why this undermodification of tRNA is present in tumor tissues, but it is not simply due to the faster growth rate of tumor cells as shown by the studies of Nishimura and Kuchino (15). The significance of this undermodification to the function of tRNAs in tumors remains uncertain. However, a recent observation by Shindo-Okada et al. (21) has provided some insight into the possible significance of undermodified tRNA species. In a study on murine erythroleukemia cells, these workers have observed that these tRNAs which normally contain the Q base in the “wobbling” position of the anticodon region have guanine inserted in its place. They have shown that, during differentiation, this guanine is gradually replaced by queueine (Q base). The reason for the hypomodification in the undifferentiated cell appears to be due to a substrate deficiency rather than to a deficiency of the specific enzyme guanine transglycosylase (insertase). Thus, it would seem that these specific modifications could be closely allied to the process of differentiation.

It would be of importance to know whether or not isoaccepting species as seen in tRNAs from Hodgkin’s tumors are also due to a change in posttranscriptional modification, as a result of a substrate deficiency. Following the isolation of pure tRNA species, it is possible that sequential analysis would help to answer such questions.

When compared with most mammalian tumors, Hodgkin’s disease has some unique problems. One of the basic difficulties is the heterogeneity of the cell population. Although controversy continues as to the true derivation of the Hodgkin’s cell, there appears to be no doubt regarding its malignancy.

The inability to establish a cell line from the “Hodgkin cell” has frustrated investigators from characterizing these cells and their properties. According to the report of Kaplan (9) “their capacity for sustained proliferation in vitro, aneuploidy and heterotransplantability establishes their neoplastic character, whereas the cell marker studies, phagocytic activity, positive staining reactions for non-specific esterase and capacity to excrete lysozyme identify them as deriving from the macrophage or other closely related cells of the mononuclear phagocyte system, rather from the lymphocyte.”

The studies reported here, coupled with the data on urinary excretory markers and methyltransferase activity, point to the uniformity of the malignant process in Hodgkin’s disease.

REFERENCES


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