Patterns of Isoaccepting Phenylalanine Transfer RNA in Human Leukemia and Lymphoma

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Summary

The patterns of isoaccepting phenylalanine tRNA (tRNAPhe) isolated from neoplastic and nonneoplastic spleens have been determined. The presence of two isoaccepting tRNAPhe's from normal human spleens has been confirmed. In addition, a third tRNAPhe could be found in tRNA extracted from malignant lymphomas and chronic leukemias. With the use of aminoacyl synthetase of human fetal liver, additional phenylalanine-accepting tRNA could be found only in patients with chronic myelogenous leukemia. Normal human spleen tRNA and tRNA derived from patients with malignant lymphoma could not be charged with phenylalanine. It is thought that a family of embryonic tRNAPhe's is present in the cells of myelogenous leukemia.

Introduction

Variation in isoaccepting tRNA's of tumor tissue as opposed to nontumorous tissue has been reported for several animal systems (6, 13, 15). Recently, Gallo and Pestka (4) have published similar findings in leukemic tissue culture cells derived from human leukemia and in cell lines derived from normal human buffy coat. The differences in isoaccepting species appear quantitative. Whether new isoaccepting species are present in neoplastic cells is still not clear.

The availability of fresh, surgically removed spleens from patients with leukemia, malignant lymphomas, and nonneoplastic disease gave us the opportunity to extract comparatively large amounts of human tRNA from these organs. Because of the homogeneity of cell types usually found in involved spleens of chronic lymphocytic and chronic myelogenous leukemia, it was hoped that a meaningful comparison of tRNA from neoplastic and nonneoplastic tissue could be made. This study is an analysis of differences in tRNAPhe extracted from various surgically excised human spleens.

Materials and Methods

The Spleens. A complete list of the splenic material used, along with diagnosis and quantity of tRNA extracted, is given in Table 1.

Method of Extraction. The freshly excised organs were washed and drained of as much blood as possible. The spleens were cut into 100-g aliquots, appropriate material was taken for histological study, and 20 g were reserved for the preparation of aminoacyl-tRNA synthesis. The remaining tissue was immediately (within 15 min) extracted by a modified Brunngraber (3) technique. To every 100 g of spleen were added 300 ml of 1.0 N NaCl, 0.05 M EDTA in 0.1 M Tris-HCl (pH 7.5), 200 µg polyvinyl sulfate, and 5% w/v sodium dodecyl sulfate. An identical volume of buffer-saturated phenol was also added, and the entire mixture was homogenized in a Waring Blender for 2 min. The pooled homogenates were centrifuged at 4° and 10,000 rpm for 10 min, and the aqueous layer was decanted. Cold 95% ethanol, 2.5 volumes, was added slowly while the mixture was briskly stirred with a glass rod. The immediately precipitating DNA was removed by winding on the glass stirring rod. The remaining solution was kept at 0° for at least 16 hr until RNA precipitation was complete.

The precipitate was pelleted by centrifugation at 10,000 rpm for 10 min at 4°. Purification of the crude tRNA precipitate was carried out as described by Zubay (17). The isopropyl alcohol precipitated tRNA was then passed through a Sephadex G-200 column as described by Anderson (1).

BD-cellulose Column Chromatography. The BD-cellulose was prepared as described by Gillam et al. (5). A column 1.5 x 55 cm was routinely used for the fractionation of splenic tRNA listed in Table 1. The salt gradient used was identical to that described by Gillam et al. In place of the 10% ethanol in salt, a reverse gradient of 30% ethanol in salt to 0% ethanol in salt was used for the elution and fractionation of phenylalanine tRNA.

Aminoacyl tRNA synthetase from spleen and human fetal liver was prepared according to the method of Hoskinson and Khorana (7). If the aminoacyl synthetase was not used immediately, it was stored in the deep freeze at -75° in the presence of glycerol.

Amino acid acceptor activity was assayed by the method of Trupin et al. (14) with 14C-labeled amino acids (Table 2).

Results

Attempts to charge the tRNA extracted from Spleens 5, 6, and 7 with a number of amino acids including phenylalanine tRNA was not successful despite stripping procedures and addition of the terminal trinucleotide according to the method of Littauer and Daniel (9). It was only after BD-cellulose

1Supported by USPHS Grant 1 MO1 RR00262-06.

2The abbreviation used is: BD-cellulose, benzoylated diethylaminoethyl cellulose.
Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
<th>tRNA charged by embryonic aminoacyl synthetase-phenylalanine-14 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's disease</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Felty's syndrome</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Giant follicular lymphoma</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia, pH chromosome negative</td>
<td>3</td>
<td>All charge</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia, pH chromosome positive</td>
<td>3</td>
<td>One charge</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>1</td>
<td>Charge</td>
</tr>
</tbody>
</table>

a The abbreviations used are: CML, chronic myelogenous leukemia; CLL, chronic lymphatic leukemia.
b tRNA extracted by the method of Rogg, Wherli, and Staehelin (12).

chromatography that these tRNA's would accept amino acids. It seems likely that some inhibitor of charging was removed or inactivated by the BD-cellulose column.

Chart 1 is a BD-cellulose fractionation of tRNA extracted from Spleen 2, obtained from a patient with Felty's syndrome, a nonneoplastic disease. Charging was carried out with a variety of amino acids with the use of isologous enzyme and aminoacyl-tRNA synthetase extracted from yeast. Isoleucine-accepting isologous tRNA's are illustrated in Chart 1. The large UV-absorbing fraction eluted with 10% ethanol contains RNA^Phe and does not accept any other amino acids. This is in clear distinction from our experience with rat liver tRNA where this fraction contains a tRNA^Ser. This has been observed by others (5).

In an attempt to resolve tRNA^Phe into its isologous amino acid-accepting tRNA's, the 10% ethanol fraction was rechromatographed on BD-cellulose (Chart 2) with a reverse ethanol gradient from 30 to 0% ethanol in 1 M NaCl (see legend to Chart 2). This system gave a good separation of 2 phenylalanine-accepting tRNA's (Chart 2).

This reverse gradient was used in place of the 10% ethanol eluate for all subsequent tRNA separations.

The tRNA extracted from Spleens 1, 2, 5, 7, 8, 11, and 12 exhibited similarities in the pattern of phenylalanine tRNA separation and charging (Chart 3).
Patterns of isoaccepting tRNA\(^{\text{Phe}}\) in Cancer

To O.D. 9.0

spleen enzyme

30 50 70 90 110 130

Tube NO.

Chart 3. BD-cellulose elution profile of tRNA Hodgkin’s disease spleen. Approximately 60 mg tRNA were fractionated with the gradients described in Charts 1 and 2. Three well-defined isoaccepting tRNA\(^{\text{Phe}}\)’s can be seen (phenylalanine\(^{14}\)C).

Two well-defined phenylalanine-accepting peaks are seen when autologous aminoacyl synthetases are used. There is a 3rd accepting area that is small but well defined (Chart 3). This phenylalanine-accepting peak elutes at a higher concentration of alcohol than the 2 major phenylalanine-accepting areas.

Attempts to charge these phenylalanine tRNA’s with the use of aminoacyl synthetases from human fetal liver were not successful.

The tRNA’s extracted from Cases 4, 6, 10, 9, and 12 exhibited a pattern of phenylalanine acceptance after BD-column fractionation that showed considerable variation. Chart 4 represents a fraction of tRNA from Spleens 4 and 6, chronic myelogenous leukemia, pH chromosome negative. These tRNA’s gave almost identical patterns of separation and phenylalanine acceptance. With autologous aminoacyl synthetases, at least 3 distinct phenylalanine-accepting tRNA’s could be identified. When human embryonic liver was the aminoacyl synthetase source, 2 new phenylalanine-accepting areas could be demonstrated.

The tRNA\(^{\text{Phe}}\)-12 acute myelogenous leukemia (Chart 5) revealed a multiplicity of phenylalanine-accepting areas when charged with the fetal aminoacyl synthetase.

The tRNA\(^{\text{Phe}}\)-9 chronic myelogenous leukemia, pH chromosome positive, demonstrated several interesting variations in phenylalanine acceptance. With autologous aminoacyl synthetase, a low level of acceptance was observed (Chart 6).

With 12-week fetus Enzyme A (aminoacyl synthetase), 3 large and 2 small areas of phenylalanine acceptance could be seen. This experiment was repeated with enzyme extracted from an older (20-week) fetus (Enzyme B). A different pattern of phenylalanine acceptance could be seen. There was only 1 major phenylalanine-accepting peak with several minor ones present.

For an investigation of the nature of these unusual phenylalanine-accepting areas revealed by fetal aminoacyl synthetases, the fluorescent properties of the phenylalanine tRNA were examined.

RajBhandary et al. (11) have commented upon the presence of an unusual base adjacent to the anticodon of tRNA\(^{\text{Phe}}\) of yeast, bovine liver, and wheat germ. This hydrophobic base fluoresces at 440 nm. Measuring the fluorescence of the fractionated tRNA\(^{\text{Phe}}\) in the Farrand spectrofluorometer enabled us to correlate fluorescence, phenylalanine acceptance, and absorbance. A reasonable concordance between phenylalanine acceptance and fluorescence was obtained (Chart 7).

Discussion

The presence of 2 isoaccepting tRNA\(^{\text{Phe}}\)’s in mammalian tissues has been reported by others (10). The finding of a 3rd isoaccepting tRNA\(^{\text{Phe}}\) in malignant lymphomas and chronic lymphatic and myelogenous leukemias has not previously been observed. The identity of any specific amino acid-accepting tRNA is dependent upon its ability to accept a radioactive amino acid. Such a system of identification is dependent upon the nature and efficiency of the aminoacyl synthetase used.
There are reports dealing with this aspect of tRNA identity (2, 8). In our system, we have used the autologous aminoacyl synthetases to identify isoaccepting tRNA^Phe^s. What surprised us was the recognition of many more tRNA^Phe^ species in myelogenous leukemia by the use of aminoacyl synthetases of human fetal origin and the varying pattern discerned depending upon the age of the fetal material. These findings were not present in nonneoplastic tRNA or in tRNA derived from Hodgkin’s disease, chronic lymphatic leukemia, or giant follicular lymphoma.

It is tempting to think that we have demonstrated the presence of a family of fetal tRNA^Phe^s in myelogenous leukemia that requires a specific fetal aminoacyl synthetase for their identity. Until tRNA^Phe^ is extracted from fetal tissues and shown to be similar to that from myelogenous leukemia, our thoughts remain conjectural. A more serious question is that of the nature of what actually has been found. Are all the isoaccepting areas truly integral tRNA^Phe^ or in part artifact? Zachou has shown that half a yeast tRNA^Phe^ may accept its amino acid. The concordance of fluorescence and phenylalanine acceptance makes artifact much less likely as a probable explanation of the multiplicity of phenylalanine-accepting areas. It is more likely that these phenylalanine-accepting areas are integral tRNA^Phe^s. Enough material is available to determine nucleoside composition and primary structure of a number of these isoaccepting tRNA^Phe^s.

REFERENCES

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