Deficiency of Queuine, a Highly Modified Purine Base, in Transfer RNAs from Primary and Metastatic Ovarian Malignant Tumors in Women

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ABSTRACT

The tRNAs from rapidly growing tissues, particularly from neoplasia, often exhibit queuine deficiency. In order to check whether different kinds of ovarian tumors display queuine deficiencies we have analyzed tRNA samples from 16 ovarian malignancies. The tRNAs from histologically normal myometrium (4 samples) and myoma (6 samples) were taken as healthy tissue and benign tumor references. Queuine deficiency was determined by an exchange assay using [8-3H]guanine and tRNA:guanine transglycosylase from Escherichia coli. The mean values of queuine deficiencies in tRNAs were: 10.95 ± 2.21 pmol/A260 in primary epithelial tumors (9 cases); 23.75 ± 7.89 pmol/A260 in primary epithelial tumors (9 cases); and 34.58 ± 7.18 pmol/A260 in metastatic tumors (2 cases). These values displayed statistically significant differences (P = 0.0003, Kruskal-Wallis test). The queuine deficiencies in tRNAs significantly increased when moving from well-differentiated through moderately differentiated to poorly differentiated tumors, with the highest values found in poorly differentiated metastatic tumors (P = 0.0002, Kruskal-Wallis test). Queuine deficiency determination in tRNAs is proposed as a factor for clinical outcome prognosis of ovarian malignancies.

INTRODUCTION

The high content of modified nucleosides is one of the characteristic features of tRNAs. These modified nucleosides are posttranscriptionally synthesized either by direct modification of a major nucleoside or by replacement of a major base by a modified one (editing) (for a review see Refs. 1 and 2). The modified nucleotides at the first position of the anticodon, the so-called “wobble position” in tRNA molecules, play an important role in fine tuning of codon-anticodon interactions in tRNA-mediated message translation from mRNA into protein chain and as determinants or antideterminants of specificity of aminoaclylation (for reviews see Refs. 1 and 3). The modifications at this position can be very simple, e.g., a single methylation, or very complicated, as it is in the case of queuine, a highly modified purine (deazaguanine derivative), which is found in isoacceptor tRNAs for asparagine, aspartic acid, histidine, and tyrosine (queuine family tRNAs) (4, 5). Queuine is incorporated into tRNA posttranscriptionally by a specific enzyme, the tRNA:guanine transglycosylase (EC 2.4.2.29), which mediates the exchange of the guanine by queuine without phosphodiester bond breakage (6). Investigations of the role of queuine in codon-anticodon interactions showed that queuine has an essential function in the enhancement of correct translation of the codons (2). In addition it has been shown that queuine-lacking tRNA-Tyr in mammalian tissues could have amber suppressor activity (7). Queuine, as a free base, is widely distributed in nature (8). However, mammals, as well as other animals, are not able to synthesize queuine on their own despite its presence in their queuine family tRNAs. In mammals, queuine is provided by the intestinal flora or as a diet factor. Furthermore, in embryonic tissues and regenerating rat liver (i.e., in rapidly growing tissues), the queuine family tRNAs are partially queuine deficient (6, 9). Similar observations of queuine deficiency have been made in queuine family tRNAs from placenta tissue despite the presence of a large amount of free queuine in the amniotic fluid (8, 10). It has in addition been observed that neoplastic tissues very often exhibit significant deficiencies in the queuine content of queuine family tRNAs (9). In induced animal tumors the queuine deficiency could be correlated with the histological signs of malignancy (9), whereas similar observations could also be done in a few cases of human malignancies, such as leukemias, lymphomas, and lung cancers (11, 12).

Our initial working hypothesis was that it might be possible that even in the early stages of the diseases the queuine content in tRNAs could already have been affected and could be determined in order to consider it as a prognostic factor. We therefore undertook investigations to estimate directly the extent of the queuine deficiency in tRNAs from various human ovarian tumors in order to check for the differences, if any, in relation to histopathological type of malignancy and grading of differentiation.

MATERIALS AND METHODS

The tissues were collected from 1983 to 1992 during the operations in the Second Clinic of Gynecological Surgery of the Medical Academy, Lublin, Poland. After surgery the samples were immediately immersed and kept in liquid nitrogen until further processing. For routine histopathological characterizations several probes from each tumor were fixed in formalin and embedded in paraffin according to standard institutional procedure (Department of Pathomorphology, Medical Academy, Lublin, Poland).

As a healthy reference, the tRNAs from histologically normal myometrium were taken because no reference sample from normal ovary could be prepared due to the tissue heterogeneity of that organ. Myoma tRNA samples were used as benign tumor controls. The reference tissues (myometrial and myoma samples) were taken from the patients who were operated for reasons other than ovarian cancer. No therapy other than surgery was applied to these patients before specimens were taken. Consent statements for further analysis of collected tumors were obtained from each patient prior to surgery. The detailed clinical data concerning these patients are presented in Table 1.

tRNA Preparation and Purification. tRNA was prepared by initial phenol extractions followed by selective isopropyl alcohol separation of the nucleic acids according to the procedure of Sein et al. (15). Crude tRNA samples were further purified on DEAE-52 cellulose. Due to DNA contamination, which was still present after chromatographic purification, bulk tRNA was additionally treated by RNase-free DNase I (Promega, Madison, WI) for 2 h at 37°C in 40 mM Tris-HCl buffer, pH 7.9, containing 10 mM NaCl and 6 mM MgCl2. After additional phenol extraction (0.5 h shaking at 4°C) and centrifugation, the water layer was collected and chromatographed on a Seph-
Table 1 Queuine deficiencies in total tRNAs from human ovarian tumors and uterus tissues

<table>
<thead>
<tr>
<th>Ovarian malignancy</th>
<th>Clinical stage (FIGO classification)</th>
<th>Tumor diameter (cm)</th>
<th>Histopathological examination (grade of differentiation)</th>
<th>Inserted [3H]guanine (pmol/A260)</th>
<th>Survival course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. T. A.</td>
<td>IA</td>
<td>5</td>
<td>Dysgerminoma (W-D)</td>
<td>12.21</td>
<td>5.0 Well (NSD)*</td>
</tr>
<tr>
<td>2. M. A.</td>
<td>IA</td>
<td>12</td>
<td>Dysgerminoma (W-D)</td>
<td>13.48</td>
<td>11.0 Well (NSD)</td>
</tr>
<tr>
<td>3. K. T.</td>
<td>IA</td>
<td>6</td>
<td>Granulosa cell tumor (W-D)</td>
<td>8.09</td>
<td>6.0 Well (NSD)</td>
</tr>
<tr>
<td>4. B. E.</td>
<td>II C</td>
<td>6</td>
<td>Granulosa cell tumor (W-D)</td>
<td>11.70</td>
<td>2.5 Well (NSD)</td>
</tr>
<tr>
<td>5. K. B.</td>
<td>IC</td>
<td>10</td>
<td>Teratoma malignatum (W-D)</td>
<td>9.25</td>
<td>Data not available</td>
</tr>
<tr>
<td>6. W. W.</td>
<td>III</td>
<td>10</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>23.74</td>
<td>1.0 Died</td>
</tr>
<tr>
<td>7. W. J.</td>
<td>III</td>
<td>10</td>
<td>Carcinoma anaplastic (P-D)</td>
<td>31.96</td>
<td>1.0 Died</td>
</tr>
<tr>
<td>8. M. H.</td>
<td>III</td>
<td>8</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>12.24</td>
<td>4.5 RD</td>
</tr>
<tr>
<td>9. M. A.</td>
<td>III</td>
<td>7</td>
<td>Serous adenocarcinoma (P-D)</td>
<td>25.63</td>
<td>0.5 Died</td>
</tr>
<tr>
<td>10. M. R. Z.</td>
<td>IV</td>
<td>8</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>35.28</td>
<td>1.5 Died</td>
</tr>
<tr>
<td>11. G. W.</td>
<td>III</td>
<td>8</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>23.75</td>
<td>4.0 Died</td>
</tr>
<tr>
<td>12. P. H.</td>
<td>III</td>
<td>10</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>23.69</td>
<td>3.0 Died</td>
</tr>
<tr>
<td>13. J. J.</td>
<td>III</td>
<td>7</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>11.03</td>
<td>3.5 RD</td>
</tr>
<tr>
<td>14. M. I.</td>
<td>III</td>
<td>9</td>
<td>Serous adenocarcinoma (M-D)</td>
<td>24.04</td>
<td>2.0 Died</td>
</tr>
<tr>
<td>15. K. M.</td>
<td>Metastatic tumor</td>
<td>5</td>
<td>Carcinoma endometriale (MP-D)</td>
<td>29.50</td>
<td>2.0 Died</td>
</tr>
<tr>
<td>16. S. K.</td>
<td>Metastatic tumor</td>
<td>12</td>
<td>Neoplasma mesenchymale prob. angiogenes (P-D)</td>
<td>39.66</td>
<td>1.5 Died</td>
</tr>
</tbody>
</table>

Reference tissues

17. L. E.                   | 38                                  | Myometrium          | 3.49                                              |
18. K. H.                   | 42                                  | Myometrium          | 3.67                                              |
19. J. M.                   | 43                                  | Myometrium          | 5.52                                              |
20. K. B.                   | 39                                  | Myometrium          | 7.15                                              |
21. K. B.                   | 39                                  | Myoma cellular       | 7.42                                              |
22. B. T.                   | 43                                  | Leiomyoma uteri     | 5.32                                              |
23. O. Z.                   | 44                                  | Leiomyoma uteri     | 6.16                                              |
24. N. M.                   | 61                                  | Leiomyoma uteri     | 6.29                                              |
25. N. M.                   | 45                                  | Leiomyoma uteri     | 3.44                                              |
26. W. A.                   | 44                                  | partim cellular      | 7.61                                              |

E. coli

Yeast (S. cerevisiae)

4.47
50.96

*= NSD, no signs of disease; RD, recurrence of disease; W-D, well differentiated; M-D, moderately differentiated; MP-D, moderately to poorly differentiated; P-D, poorly differentiated.

NSD = Not significant difference.

The same patient.

Queuine Deficiency Determination in Unfractionated tRNAs. Queuine deficiency was determined by an exchange assay using tRNA:guanine transglycosylase (6) obtained from an overproducing MRE-600 Escherichia coli strain (generous gift from Dr. K. Reuter, University of Erlangen, Nuremberg, Germany). [3H]Guanine (specific activity, 10.4 Ci/mmol) was from Sigma Chemical Co. (St. Louis, MO). The reaction mixture in a total volume of 100 μl contained 4 μg (0.1 A260) of tRNA, 2 μg (approximately 4 units) of tRNA:guanine transglycosylase, and 50 pmol (0.5 μCi) of [3H]guanine dissolved in buffer (70 mM Tris-HCl, pH 7.0-20 mM MgCl2-0.1 M NaCl-6 mM β-mercaptoethanol). Incubation time was for 1 h at 37°C. The reaction was stopped with 15% trichloroacetic acid. The precipitate was collected on Whatman No. 3MM discs and washed three times with 5% trichloroacetic acid. The sample was dried and counted by liquid scintillation (Beckman Model 6000SLC). Each tRNA sample was estimated in triplicate. E. coli and Saccharomyces cerevisiae tRNAs, both from Boehringer Mannheim (Mannheim, Germany) were used as references for either completely queuine-modified or queuine-lacking tRNAs, respectively. The results were expressed as pmol of [3H]guanine incorporated/A260 of tRNA.

Statistical analyses were performed using the Wilcoxon rank sum and Kruskal-Wallis tests.

RESULTS

The values of queuine deficiency in 16 malignant ovarian tumor tRNAs were significantly higher (range, 8.09-39.66 pmol/A260), leading to a mean ± SD of 20.95 ± 9.99 pmol/A260 as compared to normal tissue tRNAs (myometrium, 4.96 ± 1.73 pmol/A260, P = 0.0004) and benign tumors (myomas, 6.04 ± 1.53 pmol/A260, P = 0.00003). The P values were calculated using the Wilcoxon W test. There is no statistically significant difference in queuine deficiency between normal myometrium and myoma tissue tRNAs (P = 0.5). In order to find the correlation, if any, between histopathological type of tumor and queuine deficiency, the set of 16 patients with malignant ovarian tumors was divided into 3 groups. The first group consisted of 5 patients with gonadal and germ cell tumors [dysergminoma (2 cases), granulosu cell tumor (2 cases), and teratoma malignisatum (1 case)]. The mean of queuine-deficient tRNAs in this group was 10.95 ± 2.21 pmol/A260. This value was significantly lower than the mean value of the second group, which consisted of 9 patients with epithelial tumors (23.48 ± 7.89 pmol/A260, P = 0.012), and the third group, which consisted of 2 patients with metastatic tumors (34.58 ± 7.18 pmol/A260, P = 0.022) (Fig. 1).

We also examined the queuine deficiency in tRNAs as a function of grading of differentiation by dividing the cancer patients into 4 tumor groups: group 1, well differentiated; group 2, moderately differentiated; group 3, moderately to poorly differentiated; and group 4, poorly differentiated. Because we found only one case of the moderately to poorly differentiated tumor, we decided to include the corresponding value for the purpose of statistical analysis to the group of poorly differentiated tumors. This kind of classification led to the following results for queuine deficiency: group 1, 10.95 ± 2.21 pmol/A260; group 2, 21.97 ± 5.97 pmol/A260; and groups 3 and 4, 31.69 ± 8.22 pmol/A260. When analyzed by the Kruskal-Wallis test, these results indicate a significant difference between the groups (P = 0.0004). The results for queuine deficiency in tRNAs from normal myometrium and myoma tissue were similar (4.96 ± 1.73 pmol/A260 and 4.96 ± 1.73 pmol/A260, respectively, P = 0.5). In order to find the correlation, if any, between histopathological type of tumor and queuine deficiency, the set of 16 patients with malignant ovarian tumors was divided into 3 groups. The first group consisted of 5 patients with gonadal and germ cell tumors [dysergminoma (2 cases), granulosu cell tumor (2 cases), and teratoma malignisatum (1 case)]. The mean of queuine-deficient tRNAs in this group was 10.95 ± 2.21 pmol/A260. This value was significantly lower than the mean value of the second group, which consisted of 9 patients with epithelial tumors (23.48 ± 7.89 pmol/A260, P = 0.012), and the third group, which consisted of 2 patients with metastatic tumors (34.58 ± 7.18 pmol/A260, P = 0.022) (Fig. 1).
solid tumors. Among these tumors, in contrast to our results, 2 metastatic ovarian neoplasm tRNAs<sub>asp</sub> were not queuine deficient. Furthermore, Singhal and Vakharia (18) did not observe any queuine deficiency in tRNA<sub>asp</sub> from neoplasia tissue but their investigations were carried out several days after transplantation of human adenocarcinoma ovaries into athymic mice. Results obtained in our group of patients were very close to those published by Emmerich et al. (11) and Huang et al. (12). The differences between our estimations and the results of Gündüz et al. (17) and Singhal and Vakharia (18) could be a result of the different methodologies used.

The clinical outcome of ovarian malignancies depends on several factors, such as histopathological type of tumor, grade of differentiation, clinical stage recognized during laparotomy, and size of residual tumor (13, 14). The recently developed amplification of the HER-2/neu proto-oncogene (19, 20) and cancer-associated galactosyltransferase (21) could be prognostic markers in epithelial tumors [serous and clear cell (mesonephroid), respectively] but they are not suitable for prognosis of the other kinds of ovarian tumors (gonadal, germinal, and other kinds of epithelial originating tumors).

Particularly in granulosa cell tumor-diseased patients, the postoperative outcome prognosis based primarily on histopathological examination is extremely difficult. The course of granulosa cell tumor can be either slow or very rapid and progressive, even when recognized at the early stages (22). Supporting the previous observation, Scully (23) described the occurrence of two kinds of granulosa cell tumors, one being highly malignant and the other being low malignant, but prognosis of the clinical outcome could not be established histologically. In the present paper we have shown in tRNAs obtained from 2 cases of granulosa cell tumors that the queuine deficiency was low and close to queuine estimations in tRNAs from myoma tissue and almost identical to tRNAs from human placenta (10). After 2.5- and 6-year observation periods, these two granulosa cell tumor patients presented no signs of disease recurrence. The extent of queuine deficiency in tRNAs correlated with cell proliferations, and could therefore be used as a complementary factor for clinical outcome prognosis in the case of granulosa cell tumors. The problem seems to be very important for clinicians when they have to consider postoperative treatment (radiotherapy and sometimes chemotherapy) in young (i.e., reproductive age) women. However, other investigations and longer times of observation are needed to confirm these promising but only preliminary results.

Additional support for the use of the queuine content in tRNAs for the prognosis of the postoperative outcome of disease could be confirmed by further observations on several patients in epithelial and metastatic groups. Indeed, patients with the highest values of queuine-deficient tRNAs died within 1–4 years after operation (patients 6, 7, 9–12, and 14–16) but it should be noted that these patients had advanced neoplastic process; therefore, the prognosis of clinical outcome was already poor. Patients 8 (4.5 years after operation) and 13 (4 years after operation), who presented the lowest values of queuine deficiency in epithelial tumor group, are alive but are under treatment due to recurrence of the disease. Germ cell tumor patients who presented the low values of queuine deficiency in tRNAs (patients 1 and 2) did not show any signs of recurrence even 5 and 11 years after operation, respectively. At this stage of the study, the investigations could not be completed for the entire group of the patients due to the short time of observation after surgery.

The queuine-lacking tRNAs were often found in induced animal tumors and transformed cell cultures but the reason why tRNAs from tumor are hypomodified with respect to queuine nucleoside is, to date, unclear. One explanation could be the lack of tRNA:guanine transglycosylase activity in cancer cells, as reported by Gündüz et al. (17). Another suggestion comes from Katze et al. (24), who proposed that

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**DISCUSSION**

To date, only a few papers have dealt with queuine deficiency in tRNAs from human malignancies. Among them, Emmerich et al. (11) found a different extent of queuine-deficient tRNAs in human leukenias and lymphomas, which they correlated with the grade of malignancy (histopathologically and clinically considered). Similar observations were done for human lung cancer tRNAs by Huang et al. (12). The latter authors found a significant correlation between (a) queuine deficiency in tRNAs extracted from lung carcinomatous tissues, (b) grading of differentiation (histologically recognized), and (c) survival rate of the patients. Recently, Gündüz et al. (17), using a chromatographic method, found queuine-deficient tRNA<sub>asp</sub> in only 10 of 46

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**Fig. 1.** Queuine deficiency in tRNAs from normal myometrium (A), myomas (B), germ cells and gonadal tumors (C), epithelial tumors (D), and metastatic tumors (E). The differences among normal myometrium and different tumor groups are statistically significant (P = 0.0003, Kruskal-Wallis test). Columns, mean; bars, SD.

**Fig. 2.** Queuine deficiency in tRNAs from normal myometrium (I), myomas (II), well differentiated (III), moderately differentiated (IV), and moderately to poorly and poorly differentiated (V) ovarian tumors. The differences among particular groups are statistically significant (P = 0.0002, Kruskal-Wallis test). Columns, mean; bars, SD.

indicated a strong correlation between the grades of differentiation and the queuine deficiencies (Fig. 2).
queueine deficiency in tRNAs from malignant tissues could be connected with several different factors, such as: (a) low queueine uptake rate by cancer cells and higher tRNA synthesis as compared to normal cells; (b) the lack of inserting enzyme activity as already mentioned; (c) queueine competition with unknown factors (pteridines?); (d) higher catabolic rate of free queueine in cancer cells; or (e) queueine salvage deficiency in tumors, or, as Langgut and Kersten (25) found, the lack of free queueine availability for tRNA modifications in cancer cells.

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REFERENCES


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