AN INVESTIGATION OF THE MINOR BASE COMPOSITION OF TRANSFER RNA IN NORMAL HUMAN BRAIN AND MALIGNANT BRAIN TUMORS

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1. Introduction

A number of reports have described elevated levels of tRNA methylase activities in a variety of tumor tissues when compared with normal control tissues [e.g., 1–4]. Such elevated methylase activities may be due to elevated levels of the enzymes themselves or also to the absence of methylase inhibitors [5–7]. Whether changes in methylase activity are always accompanied by parallel changes in the base composition of tumor tRNA has been studied considerably less than the properties of the enzymes. Berquist and Matthews [8] have reported elevated levels of some methylated purines in tRNA from a mammary adenocarcinoma and S180 ascites tumor in mice. Viale and co-authors [9, 10] have reported substantially elevated levels of methylated purines as well as pyrimidines in tRNA from human brain tumors when compared with normal brain. Data published by Iwanami and Brown [11] for HeLa cell and L cell tRNA and by Baguley and Staehelin [12] for a transplantable rat leukemia, on the other hand, appear to be similar to minor base composition data reported for mammalian liver [12, 13]. Indirect evidence for altered tRNA species in tumors is provided by investigations of the chromatographic behavior of tRNA charged with radioactive amino acids [e.g. 14–16].

The function of the modified bases in tRNA is still rather obscure. $tRNA_I^{Gly}$ from *Staph. epidermidis* participating in peptidoglycan synthesis, but incapable of participating in protein synthesis or binding to ribosomes, contains no modified bases other than 4-thiouridine [17]. This result points to a major role of tRNA modification in the overall process of protein synthesis.

In order to answer the question of whether substantial changes in tRNA structure are generally characteristic for the neoplastic state systematic analytical studies are required which, in view of the small amounts of tissue available in many instances, have to utilize ultrasensitive methodology. Methods developed recently in this laboratory [18, 19] make use of the fact that accurate quantitative base composition analysis of RNA can be carried out by chemical incorporation of tritium label into periodate-oxidized enzymatic digests of RNA.

We have recently used this procedure in a systematic investigation of the base composition of tRNA isolated from various normal and neoplastic mammalian tissues. This communication reports on the minor base composition of tRNA isolated from normal human brain as well as from malignant brain tumors.

2. Materials and methods

Brain tumor specimens, as well as normal contiguous tissue, were obtained from Professor W.H.

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Sweet and Mrs. J. Messer, Department of Neurosurgery at the Massachusetts General Hospital. The tissues were kept on ice for less than one hour after removal and then immediately frozen between blocks of dry ice. Normal human brain tissue from the right frontal lobe of a 39-year-old patient whose death was caused by a cardiovascular disease not involving the brain was obtained from the Department of Pathology at the Massachusetts General Hospital within four hours post mortem. tRNA was isolated by direct phenol extraction [20, 21]. Yields of RNA were about 100 μg per g of normal brain tissue and about 250 μg per g of neoplastic tissue. RNA preparations were subjected to acrylamide gel electrophoresis [22] to check for impurities. RNA was digested to nucleosides as described previously [18, 19]. Aliquots of such digests were subjected to periodate oxidation and treatment with tritium labeled borohydride. Following two-dimensional thin-layer chromatography and film detection [23] the labeled nucleoside derivatives were assayed by liquid scintillation counting. The minor base composition was determined according to the equation [19]:

$$f_i = \frac{\text{cpm}_i}{\sum_{i=1}^{N} \text{cpm}_i}$$

where f_i is the base composition expressed as moles of an individual nucleoside/moles of all minor nucleosides (number = N) determined. By setting the total count rate $\sum_{i=1}^{N} \text{cpm}_i$ equal to 100 the base composition is expressed as the percentage of the total.

3. Results

Table 1 presents data on the relative distribution of twelve minor bases in unfractionated tRNA isolated from normal human brain and a glioblastoma multiforme. The normal tissue was obtained at autopsy four hours post mortem (see above). Analysis of a fresh surgical specimen (normal tissue adjacent to an astrocytoma) resulted in base distribution data identical to those presented in column 1 of table 1 [24]. The minor base composition obtained from the brain tumor specimen is almost identical to its normal counterpart (table 1). Minor differences in the values for dihydrouridine, N^2-methylguanosine, and pseudouridine appear, however, to be statistically significant. Very similar or identical base distribution data were obtained from six additional surgical specimens of glioblastoma multiforme, as well as from three specimens of astrocytoma, a more slowly growing brain tumor. Standard deviations for most minor bases are low (table 1). The higher relative standard deviation for m^6A is due to the high R_f value of m^6A trialcohol in both chromatographic dimensions resulting in a somewhat diffuse spot of this compound. Because mammalian tRNA appears to contain no m^6A [11, 25] – its presence being due to a rearrangement of m^1A [26] – the sum ($m^1A + m^6A$) probably reflects the true m^1A content of the RNA. The high standard deviation for inosine is caused by the close proximity of guanosine on the chromatographic map, which makes accurate cutting out of the inosine trialcohol spot sometimes difficult.

In table 2 our results are compared with minor base distribution data for HeLa cell tRNA reported by Iwanami and Brown [11] as well as for normal human brain and glioblastoma multiforme tRNA reported by Viale [10]. It can be seen that our results are in close agreement with data reported by Iwanami and Brown for HeLa cell tRNA. Similar results for some of these minor bases were published by Baguley and Staehelin [12] for rat liver and leukemic spleen tRNA and by Graddock [27] for rat liver and intestine as well as for rat liver after treatment with chemical carcinogens. Our data for brain tRNA also resemble the rat liver values for m^5C, m^1G, m^1A, and Ψ reported earlier by Dunn [13]. These authors have used methods completely different from our isotope derivative method, namely, labeling with methionine-methyl-^{14}C, both in tissue culture [11] and in whole animals [27], followed by chromatographic and counting techniques, and alkaline hydrolysis of nonradioactive RNA followed by chromatography and spectrophotometry [12, 13]. There is, however, disagreement between our minor base distribution data and those published by Viale and co-workers, particularly for m^1G and m^5C in normal brain tRNA, but also for other bases in normal control tissue as well as the tumor (table 2).

Table 1
Minor base composition of unfractionated tRNA from normal human brain and a malignant brain tumor (glioblastoma multiforme)*.

	Normal brain (%) ± s**	s_{rel}*** (%)	Glioblastoma multiforme (%) ± s**	s_{rel}*** (%)
m^1A	7.50 ± 0.10	1.36	7.22 ± 0.07	0.96
m^6A	1.94 ± 0.07	3.63	1.87 ± 0.12	6.42
$m^1A + m^6A$	9.44 ± 0.12	1.25	9.09 ± 0.17	1.82
hU	19.65 ± 0.37	1.88	18.07 ± 0.27	1.51
m_2^2G	4.39 ± 0.10	2.22	4.27 ± 0.15	3.55
m^2G	10.19 ± 0.25	2.43	10.89 ± 0.30	2.79
m^7G‡	3.68 ± 0.14	3.91	3.79 ± 0.19	5.13
m^1G	5.47 ± 0.22	4.04	5.30 ± 0.19	3.49
m^3C‡	1.79 ± 0.07	3.80	1.87 ± 0.04	1.91
m^5C	14.28 ± 0.21	1.45	14.13 ± 0.26	1.84
ψ	24.04 ± 0.29	1.21	25.66 ± 0.15	0.57
m^5U (rT)	4.89 ± 0.10	1.98	4.96 ± 0.07	1.31
I	2.16 ± 0.23	10.63	1.96 ± 0.25	12.71
Sum	99.98%		99.99%	

* Data are expressed as percentages of the total minor base composition.
** Standard deviation ($N = 6$).
*** Relative standard deviation $s_{rel} = s \times 100/\text{mean}$ %.
‡ Recoveries of m^7G and m^3C as tritium labeled trialcohols are about 70% and 85%, respectively. Our data are not corrected for incomplete recovery of these compounds.

Table 2
A comparison of our minor base distribution data with results reported by others for tRNA isolated from human cells *.

	HeLa cells [11]	Our data (normal brain)	Normal human brain [10]	Our data	Glioblastoma multiforme [10]	Our data
$m^1A + m^6A$	18.27	17.44	2.67	12.61	10.15	11.92
hU	n.r.**	–	n.r.**	–	n.r.**	–
m_2^2G	8.02	8.11	8.00	5.86	10.06	5.60
m^2G	15.90	18.83	4.74	13.61	16.56	14.28
m^7G	6.96	6.80	n.r.**	–	n.r.**	–
m^1G	8.67	10.11	0.30	7.31	14.07	6.95
m^3C	4.34	3.31	n.r.**	–	n.r.**	–
m^5C	28.25	26.38	1.78	19.08	6.41	18.53
ψ	n.r.**	–	74.81	32.11	34.37	33.65
m^5U (rT)	9.59	9.03	4.74	6.53	5.52	6.50
I	n.r.**	–	2.96	2.89	2.85	2.57
Sum	100.00%	100.01%	100.00%	100.00%	99.99%	100.00%

* Data of others were calculated from values published by Iwanami and Brown [11] and Viale [10]. Our data were recalculated to account for the fact that values for certain minor bases had not been reported by these authors.
** Not reported.

4. Discussion

The data reported in this communication reveal only minor differences between the overall minor base composition of normal human brain and brain tumor tRNA. In addition to differences in minor base composition there are differences in the relative proportions of the major bases between normal brain and brain tumor tRNA [28]: brain tRNA is richer in uridine than glioblastoma or normal liver tRNA. The low uridine content of glioblastoma tRNA may, however, be unrelated to the neoplastic process itself because tRNA isolated from other tumors (e.g., a chromophob adenoma of the pituitary) was found to contain relatively high amounts of uridine [28]. In no instance, thus far, have we obtained evidence for an increase in the ratio mole % of modified bases/mole% of major bases, i.e. for an actual 'hypermethylation' or 'hypermodification' of human brain tumor tRNA.

The question of whether or not alterations of tRNA are characteristic or responsible for the neoplastic transformation cannot be decided at the present time. In view of the central role of tRNA in the translation of the genetic code [e.g., 29], even minor modifications of single species may profoundly influence the pattern of protein synthesis and the response of the cell to humoral factors regulating growth.

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