ELEVATED OVERALL RATES OF TRANSMETHYLATION IN CELL LINES FROM DIVERSE HUMAN TUMORS

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(Received 2 August, 1984; editor G. H. Sato)

SUMMARY

In a study of a diverse set of human tumor cell lines previously shown to all have a
defect in methionine metabolism (Stern, P. H., Wallace, C. D. and Hoffman, R. M., J.
Cellular Physiology 119, 29–34, 1984), we demonstrate in this report that all have
enhanced overall rates of transmethylation compared to normal human fibroblasts.
Transmethylation rates were measured by blocking S-adenosylhomocysteine hydrolase
and measuring the AdoHey which accumulates as a result of transmethylation. The
enhanced transmethylation rates may be the basis of the above-mentioned defects in
methionine metabolism previously reported in human tumor cells, including the basis of
the inability of the majority of the tumor cells to grow when methionine is replaced by
homocysteine. The excess and unbalanced tRNA methylation observed for the last 25
years in many types of cancer may be at least in part explained by our results of
elevated rates of overall transmethylation in cancer cells. The alteration of such a
fundamental process as transmethylation in cancer may be indicative of its importance
in the oncogenic process.

Key words: S-adenosylhomocysteine; S-adenosylmethionine; oxidized 3-deazaadenosine;
methionine dependence.

INTRODUCTION

Methionine dependence is a metabolic defect seen in a wide variety of transformed and malign-
nant human and animal cells in culture, and has never been found in normal cell strains (1,2).
This defect is defined as the inability or reduced ability compared to normal cells to grow in
culture medium where methionine (Met) has been replaced by its immediate precursor homocysteine
(Hcy), a medium which has been termed Met"Hcy".

In a study of 23 cell lines derived from diverse human tumors, we have recently found
the majority to be methionine-dependent (2). The distinguishing feature between methionine-dependent
and methionine-independent cells, those able to grow in Met"Hcy" medium, is the inability of methionine-dependent cells to generate sufficiently high amounts of S-adenosylmethionine
(AdoMet) compared to S-adenosylhomocysteine (AdoHey) when incubated in Met"Hcy" medium
(3,4,5). Importantly, however, both the methionine-independent and methionine-dependent tu-
mor cell lines exhibit very low pool sizes of free Met compared to normal cells incubated in
Met"Hcy" medium (5). These data suggested that cancer cells in culture may universally have
a metabolic defect in methionine/methylation metabolism that frequently but not always results
in methionine dependence. The excess and unbalanced tRNA methylation observed for the last
25 years in many types of cancer (32) may be also related to the observed defect in methionine/
methylation metabolism. The elucidation of this defect in methionine/transmethylation metabolism
is the subject of this report. An outline of methionine/methylation metabolism appears in
Figure 1. For a recent review of altered methionine/transmethylation metabolism in cancer, see
reference 31.
Fig. 1. Pathway of methionine/transmethylation metabolism.

Materials and Methods

Cell lines. The human tumor cell lines used in this study are listed in Table 1 which indicates the origin of the cells. Normal cell strains used are human foreskin FS-3, FS-5 and BA kindly donated by Professor J. A. Schneider and adult skin RO kindly donated by Professor S. Mendoza.

Media. All except the following cells were grown in Eagle’s minimum essential medium (MEM) supplemented with 10% fetal bovine serum: A498, HT1080, A204, and A2182, which were grown in Dulbecco’s modified MEM supplemented with 15% fetal bovine serum. All media were supplemented with 100 μM folic acid and 1.5 μM hydroxocobalamin.

Radioactive compounds. [35S]Met was purchased from Amersham (Arlington Heights, IL) at a specific activity of 1,420 Ci/mmol (1 Ci = 3.7 × 10^10 becquerels). [35S]Met was synthesized by a modification of the procedure of Baernstein (6). [35S]Met was isolated Met-free by passage through an alumina column which retains the Met because of interaction with the carboxyl group but does not retain Met thiolactone (7).
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same concentration of unlabeled Met. After 24 hours of labeling, freshly prepared periodate-oxidized 3-deazaadenosine (Figure 2) was added to give a final concentration of 10 μM (8), an amount which we have demonstrated to block the hydrolysis of AdoHcy (see Figure 3). The oxidized 3-deazaadenosine was prepared as follows: 10 μl of an 0.1 M solution of sodium periodate in water was added to 90 μl of an 11 mM solution of 3-deazaadenosine in 0.11 M acetate buffer, pH 4. The mixture was allowed to stand protected from light for 1 hour at room temperature and was then added to 9.9 ml ice-cold cell culture medium. In the presence of the inhibitor, the time-dependent accumulation of AdoHcy was measured as the indicator of the rate of AdoMet-dependent transmethylation. A point was measured each half hour for a period of 2½ hours.

To measure the transmethylation rate in Met Hcy medium, similar procedures were followed except that the [35S]Met medium was replaced with 1 ml Met Hcy medium containing 25 μCi 100 μM [35S]Hcy one hour prior to addition of the inhibitor. The time-dependent accumulation was measured as described above.

**Measurement of AdoMet and AdoHcy.** The cells were cooled on ice, the medium removed and the cells were washed two times with ice-cold phosphate-buffered saline. Added to each

**FIG. 2.** Structure of AdoHcy hydrolase inhibitor periodate-oxidized 3-deazaadenosine prepared by reaction of 3-deazaadenosine with sodium periodate at room temperature in 0.1 M acetate, pH 4.

**FIG. 3.** Inhibition of AdoHcy hydrolysis in neuroblastoma SK-N-SH cells by periodate-oxidized 3-deazaadenosine. Cells were incubated for 24 h in the presence of varying concentrations of the inhibitor. AdoHcy was measured as described in Materials and Methods. Complete inhibition was assumed to have occurred when further increase in inhibitor concentration produced no further change in AdoHcy concentration.
35 mm cell-culture dish were 75 µl of ice-cold 5% sulfosalicylic acid in which the cells were extracted for a minimum of one hour. Each dish was scraped with a rubber policeman. The liquid and the dislodged particulate cellular material were removed and placed in an ice-cold Eppendorf tube. The tubes were centrifuged at 4° C for 5 minutes in a Beckman microfuge. The supernatant was then used for liquid-chromatography analysis of AdoMet and AdoHcy.

To isolate [35S]-labeled AdoMet and AdoHcy, an Altex programmable liquid chromatography unit was used with a Vydac strong-cation exchange column (Separations Group, Hesperia, CA). The method (3) is a modification of that used by Chiang, Cantoni and coworkers (9,10). Briefly, a measured aliquot of the above-described cell extract was injected into the machine, which was set at a flow rate of 2.5 ml min. For the first 10 minutes, the material was eluted with a mixture of 0.01 M ammonium formate (pH 4) and a linear gradient of 0.8 M ammonium formate (pH 4) increasing from 0 to 15% of the total solution. For the next 10 minutes the percentage of 0.8 M buffer was increased linearly to 20% and then to 100% over the next 5 minutes. The percentage of 0.8 M buffer was maintained at 100% for the remainder of the run, which was terminated at 35 minutes from the time of injection. The machine was calibrated with authentic AdoHcy, which eluted between minutes 14 and 20, when 1.25 ml fractions were collected, and with authentic AdoMet, which eluted between minutes 29 and 35, when 2.5 ml fractions were collected. The radioactivity of each fraction was then measured on a Beckman LS 100 scintillation counter using a toluene-based scintillation fluid to quantify the [35S]-AdoMet and [35S]AdoHcy. Proteins were measured by the method of Lowry et al. (11).

RESULTS

Efficacy of oxidized 3-deazadenosine to block AdoHcy hydrolysis. In order to use the accumulation of AdoHcy as a measure of the overall rates of transmethylation, its hydrolysis must be blocked by inhibiting AdoHcy hydrolyase. Periodate-oxidized 3-deazadenosine was chosen as an inhibitor because it has been demonstrated to block the AdoHcy hydrolyase without serving as a substrate (12). Figure 3 demonstrates the efficacy of this inhibitor in preventing the hydrolysis of [35S]AdoHcy in human neuroblastoma SK-N-SH cells previously labeled with [35S]Met. As can be seen from Figure 3, maximum inhibition seems to be reached at 1 µM inhibitor.

To ensure complete inhibition for all cell lines, 10 µM periodate-oxidized 3-deazaadenosine was chosen for our experimental conditions, since this concentration is still orders of magnitude below that which might affect ribonucleoside reductase (13).

Rates of transmethylation in tumor and normal cells. To measure the overall rates of cellular transmethylation, the following strategy was followed: (1) AdoHcy hydrolysis was blocked by periodate-oxidized 3-deazaadenosine; (2) With AdoHcy hydrolysis blocked, the time-dependent accumulation of AdoHcy should reflect the rate of transfer of methyl groups from AdoMet and thereby its conversion to AdoHcy; (3) Since transmethylation rates are dependent on amounts of AdoMet, and inversely proportional to the amount of AdoHcy accumulated, rates were normalized by multiplying the amount of cellular AdoHcy present by the AdoHcy/AdoMet ratio present at each time point measured. Therefore, transmethylation rates could be directly compared in the various cell types normalized at each time point for their AdoMet and AdoHcy amounts.

Figure 4 demonstrates the transmethylation rates in 18 human tumor cell lines and 4 normal human fibroblast cell strains. It can be seen in Figure 4 that essentially all of the human tumor cell lines tested have rates of transmethylation that are greater than those of normal fibroblasts FS-3, FS-5, BA and RO. The rate enhancement varies from a relatively low factor for rhabdomyosarcoma A204, lung cancer cell lines SK-LU and A2182, fibrosarcoma 8387, osteogenic sarcoma HOS, neuroblastoma SK-N-SH and kidney carcinoma A498, all the way to a factor of 12 for DU145. There is no correlation between methionine-dependence and transmethylation rates, as the tumor cell lines are both methionine-dependent and independent. This agrees with previous results which show reduced free-methionine pools for all tumor cell lines, whether methionine-dependent or independent (5).

When the transmethylation rates are normalized for cellular contents of AdoMet and AdoHcy, the difference between the tumor cells and normal cells becomes even greater (Figure 5), implying a difference in overall transmethylease activity between the normal and tumor cells that is
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FIG. 4. Rates of transmethylation of human tumor cell lines and normal human fibroblast cell strains. All cells, with the exception of malignant melanoma A375 and rhabdomyosarcoma A204 were plated for 24 hrs at a density of 10^4 cells/35 mm petri dish. A375 and A204 were plated at a density of 2.5 x 10^5 cells/60 mm petri dish. All cells were labeled with 100 μM [3H]-methionine-containing medium (25 μCi/ml) for 24 h. Periodate-oxidized 3-deazaadenosine was added to a concentration of 10 μM and the accumulation of [3H]AdoHcy was measured at half-hour intervals for 2½ hours as described in Materials and Methods. Values are counts/min 3H/μg cellular protein. Solid lines are human cancer cell lines. Dashed lines are normal cell strains. See Table 1 and Materials and Methods for origin of cell types.

independent of the amounts of AdoMet and AdoHcy present.

Most of the tumor cell lines also have enhanced rates of transmethylation compared to the normal fibroblasts in Met/Hcy- medium in the presence of periodate-oxidized 3-deazaadenosine (data not shown).

When the normal FS-3 fibroblasts were plated at one quarter the density that they were for the experiments in Figures 4 and 5, their rate of transmethylation rose only slightly (data not shown). The cells plated at the lower density were dividing considerably more than the cells plated at the higher density. These experiments demonstrate that growth rate does not greatly influence the transmethylation rate of these cells.

DISCUSSION

It has been demonstrated that a number of human and animal tumors have elevated levels of tRNA methyltransferase (14), an excess and unbalanced tRNA methylation (32) and elevated levels of methylated bases excreted (15). It would seem that enhanced transmethylation rates in the cancer cells we have observed are responsible at
Fig. 5. Rates of transmethylation of human tumor cell lines and normal human fibroblast cell strains as measured in Figure 4 but corrected for varying pool sizes of AdoMet and AdoHcy by multiplying the values from Figure 4 by the ratio of AdoHcy/AdoMet at each time point. Values are counts/min ^3H/mg cellular protein. Solid lines are human cancer cell lines. Dashed lines are human normal cell strains. See Table 1 and Materials and Methods for origin of cell types.

least in part for the unbalanced transmethylation occurring in cancer. Possibly certain transmethylation are enhanced at the expense of others. For example, we have found that many of the same tumor cell lines characterized in this report have deficient DNA methylation (16). It is noteworthy that immortalized lymphoblasts which may be considered partially oncogenically transformed have higher rates of transmethylation than stimulated or quiescent lymphoblasts (17). The elevated rates of transmethylation in cancer cells may also be responsible for the high percentage of cancer cells that are methionine-dependent. It is quite possible that cells which cannot compensate for high transmethylation rates have low AdoMet/AdoHcy ratios in Met-Hcy medium
and therefore do not grow in this medium. Cancer cells that can regulate the AdoMet/AdoHcy ratio despite high transmethylation rates can grow in Met′Hcy′ medium. This hypothesis is given support by the fact that cancer cells which have reverted from methionine dependence to methionine independence still have high rates of transmethylation but the revertants have increased their AdoMet/AdoHcy ratio in Met′Hcy′ media (3) by an as yet-to-be-determined compensation mechanism (manuscript in preparation). Thus, the cancer cells that have reverted from methionine dependence to methionine independence resemble the "wild-type" methionine-independent cancer cells (5) and not normal methionine-independent cells.

The alteration of such a fundamental process as transmethylation in cancer may be indicative of its importance in the oncogenic process.

REFERENCES


This study was supported by grants 1348A and 1496R1 from the Council for Tobacco Research-USA, Inc. grant CA27564 from the National Cancer Institute, and Research Career Development Award CA00804 from the National Cancer Institute, all to Robert M. Hoffman, and by the George A. Jacobs Memorial Fund for Cancer Research. We thank Dr. P. K. Chiang for his generous gift of 3-deazaadenosine.
EDITOR'S STATEMENT

This report describes increased rates of transmethylation in a large number of human tumor cell lines in culture, compared to transmethylation rates of several strains of untransformed human fibroblasts. All studies of this kind, using tumor cell lines of epithelial origin and employing as controls “normal” (untransformed) cell strains that are solely of fibroblastic origin, are difficult to interpret and remain open to question. However, the authors' observations that cell lines derived from both sarcomas and carcinomas exhibit enhanced transmethylation rates may strengthen the case somewhat. More importantly, the potential relationship discussed by the authors of enhanced transmethylation rates to the phenomena of methionine dependence and unbalanced tRNA methylation make the data presented worthy of note.

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