METHIONINE DEPENDENCE IN CANCER CELLS — A REVIEW

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(Received May 26, 1981; accepted October 16, 1981)

SUMMARY

Methionine dependence is a defect found in many cancer cell lines that inhibits their growth in culture when methionine is replaced by its immediate precursor, homocysteine, in the culture medium. Normal cultured cells do not have this defect. This report lists the diverse and large number of animal and human cancer lines that are methionine-dependent, and critically reviews the cell biology and methionine biochemistry of the phenomenon.

Key words: altered growth response in cancer cells; altered biochemistry in cancer cells.

Methionine dependence is defined here as: (a) the inability, or reduced ability, with respect to normal cells of many different kinds of cultured cancer cells, to proliferate when methionine (Met) is replaced by its immediate precursor homocysteine (Hcy) in the growth medium (Met•Hcy• medium) or (b) the greater-than-normal reduced ability of cancer cells to grow on Met•Hcy• medium compared to their ability to grow on Met•Hcy• medium, or both of these. Cancerous cell lines reviewed here enter either or both aspects of the phenomenon of methionine dependence. Figure 1 (Hoffman, R.; Jacobsen, S., unpublished data) shows the following types of cells growing in Met•Hcy• medium: normal human fibroblasts, SV40-transformed human fibroblasts, and variants or revertants of SV40-transformed fibroblasts selected to grow in Met•Hcy• medium. As one can see, each fully transformed line behaves differently and not all are methionine-dependent by Definition a. However, if both definitions are considered, all the fully transformed cell lines in Fig. 1 may be considered methionine-dependent. As can be seen in Table 1, the types of cells exhibiting the methionine-dependent phenotype as defined above are quite diverse and include leukemias, lymphomas, hepatomas, lung carcinomas, breast carcinomas, neuroblastomas, and bladder carcinomas of human and animal origin. These facts suggest an important relationship of methionine dependence to oncogenic transformation itself in many cases and a review and further understanding of the phenomena involved seems currently highly pertinent.

Biochemical Studies

The major elements of metabolism of methionine and related molecules is illustrated in Fig. 2. As can be seen, Hcy can be methylated to form Met in two ways. One reaction is catalyzed by 5-methyltetrahydropteroyl-L-glutamate: L-homocysteine S-methyltransferase (EC 2.1.1.13), which will be referred to in this paper as methionine synthetase. This reaction requires vitamin B₁₂ and S-adenosylmethionine (SAM) as cofactors and 5-methyltetrahydropteroyl-L-glutamate (5-CH₃H,PteGlu) as the methyl donor (1). The other reaction is catalyzed by betaine: homocysteine methyltransferase (2). It is the first pathway that operates in cultured cells under usual laboratory conditions.

In 1973, in the context of a study of 5-CH₃H,PteGlu utilization, Chello and Bertino (3) reported that the L5178 mouse leukemia line required methionine supplementation for optimum cell growth even in the presence of 5-CH₃H,PteGlu, B₁₂, transcobalamin II, and Hcy. This is in contrast to normal cells whose Met requirement can be satisfied by replacement of methionine with Hcy and vitamin B₁₂. No explanation was given by Chello and Bertino as to why L5178Y has an absolute requirement for preformed Met. Halpern et al. (4) furthered the work of Chello and Bertino by showing that cell lines of
FIG. 1. Growth of normal and oncogenically transformed human fibroblasts in Met·Hey' medium. Normal cells are MGF323, MGF316, MGF292, AF2, and WI38. O—O—O. Oncogenically transformed cells are SV40-human fibroblasts and include WI38VA13, GM637, P1, and P5. —■—■—. Methionine-independent revertants of P1 and P5 are R1 and R5, respectively. ▲—▲—▲.

the Walker-256 (W-256) breast carcinocarcinoma of rats, L1210 lymphatic leukemia of mice, and the J111 monocytic leukemia of humans did not grow in McCoy's medium when Met was replaced by 15 mg/l L-Hey. Varying amounts of vitamin B₁₂, folic acid, Hey, choline, serine, or histidine did not permit growth of these lines in the absence of Met. Importantly, the addition of Hey (0.1 mm) to the medium containing Met (0.1 mm) had no effect on the growth of the cells demonstrating that Hey is not toxic. In contrast, these authors found that rat liver fibroblasts, human breast fibroblasts, and human prostate fibroblasts grew essentially as well in Met·Hey' medium as in Met·Hey medium.

The Halpern group (5) measured the methionine synthetase activity in extracts of methionine-independent and methionine-dependent cells. Cells were grown in medium containing Met, folic acid, and vitamin B₁₂. For normal methionine-independent rat fibroblast line L3-16 activities, in nanomoles Met formed per milligram protein per hour, were 32 in the presence of cyan-o-B₁₂ in the assay medium and 23 in absence of B₁₂. For methionine-independent rat thymus fibroblasts the activities were 34 in the presence of B₁₂ and 26 in its absence. For the following methionine-dependent cells the activities were as follows: L1210, 1.5 in presence of B₁₂ and 0.6 in the absence; W-256, 7.7 in presence of B₁₂, and 3.3 in its absence; for J111, 27 in the presence and 7.8 in the absence.

Measurements of methionine synthetase in the absence of B₁₂, (cyanocobalamin) in the assay medium have been taken as a measure of the amount of methionine synthetase holoenzyme. Thus the Halpern group (5) has shown that the percentage of methionine synthetase in the holoenzyme form (activity in absence of B₁₂/activity in presence of B₁₂ × 100) is about 75% for these normal cells but less than 40% for the methionine-dependent cancer cells. It is suggested (5) that since the J111 cell line has high methionine synthetase activity when B₁₂ is added to the assay medium they may be defective in the transport of B₁₂ into the cell.

TABLE 1
MALIGNANT AND TRANSFORMED CELL LINES THAT ARE METHIONINE-DEPENDENT

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker-256 (rat breast carcinocarcinoma)</td>
<td>4, 7</td>
</tr>
<tr>
<td>SV80 (human SV40 transformed)</td>
<td>7</td>
</tr>
<tr>
<td>WI38A2 (human SV40 transformed)</td>
<td>7</td>
</tr>
<tr>
<td>LS178Y (mouse leukemia)</td>
<td>3</td>
</tr>
<tr>
<td>L1210 (mouse lymphatic leukemia)</td>
<td>4</td>
</tr>
<tr>
<td>SV-BHK-21 Syrian hamster (SV40 transformed)</td>
<td>6</td>
</tr>
<tr>
<td>LM (TK⁺) (mouse L cell)</td>
<td>33</td>
</tr>
<tr>
<td>A9 (mouse L cell)</td>
<td>33</td>
</tr>
<tr>
<td>HTC (rat hepatoma)</td>
<td>35</td>
</tr>
<tr>
<td>G7G-11 (rat hepatoma)</td>
<td>33</td>
</tr>
<tr>
<td>B16 (mouse melanoma)</td>
<td>33</td>
</tr>
<tr>
<td>MMT (mouse mammary tumor)</td>
<td>33</td>
</tr>
<tr>
<td>RAG (mouse renal adenocarcinoma)</td>
<td>35</td>
</tr>
<tr>
<td>C6 (rat glioblastoma)</td>
<td>33</td>
</tr>
<tr>
<td>SV eye (mouse)</td>
<td>33</td>
</tr>
<tr>
<td>TRL 2 (rat liver transformed epithelial cell)</td>
<td>12</td>
</tr>
<tr>
<td>TRL 8 (rat liver transformed epithelial cell)</td>
<td>12</td>
</tr>
<tr>
<td>AT2 (rat liver transformed epithelial cell)</td>
<td>12</td>
</tr>
<tr>
<td>F815 (mouse mastocytoma)</td>
<td>21</td>
</tr>
<tr>
<td>A-549 (human lung adenocarcinoma)</td>
<td>21</td>
</tr>
<tr>
<td>CCRF-HSB-2 (human acute lymphoblastic leukemia)</td>
<td>21</td>
</tr>
<tr>
<td>CCRF-SB (human acute lymphoblastic leukemia)</td>
<td>20</td>
</tr>
<tr>
<td>SK-BR-2-2 (human adenocarcinoma of the breast)</td>
<td>20</td>
</tr>
<tr>
<td>SK-N-MC (human neuroblastoma)</td>
<td>20</td>
</tr>
<tr>
<td>RJ (human bladder carcinoma)</td>
<td>15</td>
</tr>
<tr>
<td>MB (mouse bladder carcinoma)</td>
<td>15</td>
</tr>
<tr>
<td>TLX5 (mouse lymphoma)</td>
<td>14</td>
</tr>
</tbody>
</table>
That is, they have large amounts of apo-enzyme, but even when grown in medium rich in B12, they cannot transport it to form holoenzyme. The L1210 and W-256 cells were said possibly to have any one of several defects as their extracts demonstrate very small amounts of apo- and holo-enzyme. No attempt was made (5) to measure any aspects of in vivo methionine or folate metabolism to see if there was correlation between in vivo and in vitro results.

Jacobson et al. (6) compared normal methionine-independent BHK-21 and the SV40-transformed derivative BHK-21-A8, which they showed to be methionine-dependent. That is, Eagle's MEM medium deficient in Met but containing folic acid, B12, and Hcy supported vigorous growth of BHK-21 but no growth of BHK-21-A8. The A-8 cells survived slightly better when 5-CH3H3PteGlu was substituted for folic acid. Enzyme studies demonstrated that the activity of 5,10-methylenetetrahydrofolate reductase, the enzyme catalyzing the synthesis of 5-CH3H3PteGlu was 42.9 nmol converted per hour per milligram protein in BHK-21 as compared to 3.2 in BHK-21-A8, and methionine synthetase was 76.2 nmol converted per milligram protein per hour for the normal cells compared to 3.7 for the transformed cells. All these measurements were for cells incubated in Met Hcy' medium containing B12. It was concluded that the methionine dependence of BHK-21-A8 was possibly due to a deficiency of the reductase. However, even large amounts of the product of the reductase, 5-CH3H3PteGlu did not support growth of the BHK-21-A8 cells. This could be due to the reduced amount of the methionine synthetase in BHK-21-A8 cells, but the reduced amount compares to normal amounts found in Halpern's study. No in vivo data were presented in this study.

Hoffman and Erbe (7) demonstrated that SV40-transformed human fibroblasts lines SV80 and W18VA2 are methionine-dependent. However, with these two cell types and the W-256 cells three lines of evidence indicated that the transformed and malignant cells synthesize large amounts of Met through methionine synthetase:

(a) The activities of the methionine synthetase were comparable in extracts of malignant and normal cells. For cells grown in the presence of B12, and assayed with B12, activities were for methionine-independent normal human fibroblasts MGF323 and MGF316, 4.93 and 12.3, respectively, and for the methionine-dependent cells W-256, SV80, and W18VA2, 4.75, 9.14, and
6.92, respectively. When these extracts were assayed in the absence of B, the activities were for MGF323 and MGF316, 3.72 and 10.4, respectively, and for W-256, SV80, and W18VA2, 1.96, 4.12, and 3.27, respectively.

(b) The uptake of radioactive label from [5-\textsuperscript{14}C]-CH\textsubscript{3}H\textsubscript{2}PteGlu, the methyl donor for methionine synthetase, was at least as great in the malignant and transformed cells and much greater in SV80 than in the normal cells. It was nearly totally dependent on the addition of Hcy, the methyl acceptor; furthermore, 39 to 84% of the label incorporated by the cells was recovered as Met.

(c) Although the malignant and transformed cells were unable to grow in Hcy alone, in the presence of otherwise limiting amounts of exogenous Met, Hcy greatly stimulated the growth of these cells.

Importantly, in the absence of Hcy, the minimum concentration of Met necessary to initiate growth of normal and of malignant and transformed cells was approximately the same, indicating that the methionine-dependent cells didn’t have a greater intrinsic requirement for Met (7) (Fig. 3).

Therefore, the results of Hoffman and Erbe (7) suggest that Met is synthesized at high rates in the methionine-dependent cells that they investigated. The actual free methionine pools were not measured in these cells. These data, however, suggest a defect other than inability to synthesize Met in at least these cells.

Hoffman et al. (8) isolated rare cells from the W-256 and the two human transformed lines above that have spontaneously regained the ability to grow in Met/Hcy medium. Six revertant lines, one from W-256 and five from SV40-transformed human fibroblasts, were characterized for three measures of methionine biosynthetic capacity: (a) methionine synthetase in cell extracts, (b) methylenetetrahydrofolate reductase activities in cell extracts, and (c) uptake of label from [5-\textsuperscript{14}C]-CH\textsubscript{3}H\textsubscript{2}PteGlu by intact cells. When all three measures of methionine biosynthetic capacity are considered, two revertants isolated from SV40-transformed cells regained the ability to grow like normal cells in Met/Hcy medium without substantial changes in these measures, including the percent of methionine synthetase in the holoenzyme form. Thus, increased methionine biosynthesis was shown not to be a prerequisite for reversion from methionine dependence to independence, and these data suggest that the original defect is not in methionine biosynthesis as was previously thought. Other revertants, however, had increased levels of one or more of the activities measured, possibly correcting the parental defect by compensation.

Subsequent studies (9-11) have confirmed that methionine synthetase activity in at least some methionine-dependent and methionine-independent cells are comparable. In fact, Tisdale (11) reported a sixfold induction of methionine synthetase in methionine-dependent cells in Met/Hcy medium.

Poirier and Wilson (12,13) have demonstrated that transformed rat liver fibroblasts have a heterogeneous behavior in Met/Hcy medium with some cell lines barely growing and others growing well as Fig. 1 demonstrates for the human SV40-transformed fibroblasts. They point out that the difference in growth rate or growth constant defined as

\[ K = \ln \frac{n_f}{n_i} / t_f - t_i. \]

where \( n_f \) = the final number of cells, \( n_i \) = initial number of cells, \( t_f \) and \( t_i \) = the final and initial days at which \( n_f \) and \( n_i \) were taken, respectively, in Met/Hcy medium with respect to Met/Hcy medium, for each cell line best differentiates their

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**Fig. 3.** Growth rate of normal and SV40-human fibroblasts and the W-256 rat breast carcinoma as a function of methionine concentration in the growth medium. No homocysteine is present. Normal cells include MGF323 and MGF316; SV40-transformed cells include W18VA2 and SV80.
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They mention that the decrease in growth rates observed with the transformed lines seems to be due to their faster growth rates in Met-Hcy medium rather than to their slower growth in Met-Hcy medium. This implies that there is no specific defect in the slow growing transformed lines. This certainly does not appear to be the case in the human SV40-transformed cell lines and the rat W-256 cell line, which grow poorly in Met Hcy medium, because as mentioned above revertants can be isolated from these cells that grow very rapidly in Met Hcy medium (Fig. 1 and reference 8) with the revertants seemingly correcting a defect.

These same authors go on to point out (13) a rough correlation between rate of growth in Met Hcy medium and cellular levels of methionine synthetase for 16 normal and transformed lines including theirs and those studied by Halpern et al. (4) and Hoffman and Erbe (7). They suggest that methionine synthetase appears to be a major determinant for growth in Met Hcy medium. However, these authors ignore the results of Hoffman et al. (8) demonstrating that revertants of transformed cells retaining the ability to grow in Met Hcy medium can be isolated with basically no change in methionine synthetase. Therefore, it seems that, although a number of lines of evidence indicate that factors in addition to methionine synthetase may determine the ability of at least some cell lines to grow in Met Hcy medium, the level of methionine synthetase may be a critical factor.

Both Poirier and Wilson (13) and Tisdale in particular (11,14,15) suggest, as alluded to above, that methionine dependence is not due to any innate biochemical defect and may be due to a higher methionine requirement, possibly due to enhanced methylating capacity of the tumor cells. However, as Hoffman and Erbe (7) reported and as demonstrated in Fig. 3 (Hoffman, R., unpublished results) for human cells, at least, there is no major difference between normal and methionine-independent transformed cells in their intrinsic methionine requirement for growth in the absence of homocysteine. Much work remains to be done although, as described below, some interesting data are accumulating that may lead to a much clearer understanding.

Another aspect of the growth arrest of methionine-dependent cells was demonstrated by Hoffman and Jacobsen (16). They have shown when SV40-transformed human cells are incubated in Met Hcy medium the cells reversibly arrest in the late-S/G2 region of the cell cycle.

Tisdale (14,17) reported that in Met Hcy medium the methionine-dependent W-256 cells demonstrated possible reduced levels of S-adenosylmethionine (SAM), the universal methyl group donor, and growth ceases in Met Hcy medium possibly due to the accumulation of methyl-deficient nucleic acid. Perhaps undermethylation of nucleic acids causes the late-S/G2 arrest. Tisdale’s data indicate that protein synthesis is not affected when Met is replaced by Hcy in the W-256 cells, which indicates that endogeneously-synthesized Met is available for protein synthesis but possibly not available in sufficient amounts for enough SAM to supply all the methylation requirements of the cell.

In summary, the basic biochemical defect of methionine dependence has not been solved. Although the data and interpretations in the literature are somewhat conflicting, it appears that at least some methionine-dependent cells methionine synthetase is normal. However, in these cells the size of the free-methionine pool has not been measured and compared to normal cells in both methionine-containing medium as well as in Met Hcy medium. Tisdale (14,17) has begun important work on the measurement of SAM, which is a key metabolite of Met and the universal methyl donor and may be in short supply when the cells are shifted from Met Hcy to Met Hcy medium. However, the levels of SAM must be compared in both types of media in closely related pairs of methionine-dependent and methionine-independent cells and compared to the amount of free methionine. The levels of S-adenosylhomocysteine (SAH) a universal inhibitor of methylation reactions must also be measured under these conditions. In other systems (24), it has been shown that when the SAH-to-SAM ratio reaches a certain level, methylation of key macromolecules in the cell is greatly reduced and cell division ceases. It must also be emphasized that SAH can be synthesized directly from homocysteine and adenosine (Fig. 2).

Relationship of Methionine Dependence to Oncogenic Transformation

An important question raised by the above studies is whether methionine dependence is a basic aspect of oncogenic transformation. In addition to the large and diverse number of cancer
cells displaying the methionine-dependent phenotype, further evidence for the linkage of methionine dependence and oncogenic transformation comes from studies of cells that have reverted from methionine dependence to methionine independence. Hoffman et al. (18) have shown that the methionine-independent revertants of both SV40-transformed human fibroblasts and the W-256 rat malignant cells have simultaneously reverted for some of the properties associated with the transformed state. Of the 13 methionine-independent revertants described, five showed increased anchorage dependence, eight showed an increased serum requirement for optimal growth, eight showed decreased cell density in medium containing high serum, and three altered their cell morphology significantly. Eight of the thirteen have increased chromosome numbers. Thus by selecting for methionine independence it is possible to select for heterogeneous transformation revertants indicating further an intrinsic relationship between altered methionine metabolism and oncogenic transformation. The exact nature of this relationship remains an important, yet unanswered, question.

In Vivo Studies

Koziorowska et al. (19) have performed important experiments whereby they demonstrate the methionine dependence of both rat sarcoma and murine leukemia cells taken directly from the animal and grown in culture. Thus, methionine dependence is an in vivo as well as an in vitro phenomenon. More of these studies must be done particularly with human tumors.

Possibilities for Therapy Based on the Methionine-Dependent Phenotype of Tumor Cells

The fact that normal and many cancer cells grow so differently in Met-Hcy medium suggests a possibility of using this phenomenon as a basis for a new type of tumor therapy. In fact, experiments toward this goal were undertaken by Kreis et al. (20-22), who have found in cell cultures that the enzyme methionase (L-methionine-ð-deaminor-mercapto methane-lyase EC4.4.1.11) at a concentration of 0.1 U/ml can lead to a cessation of cell growth. However, Hcy in medium containing L-methionase could partly "rescue" the normal methionine-independent cells but not the malignant methionine-dependent ones. In mixed cultures of methionine-independent and methionine-dependent cells with added methionase and Hcy the normal fibroblasts grew and synthesized DNA vigorously whereas the malignant methionine-dependent cells lost their viability within 3 to 4 d (20,21).

Thus there exists the possibility of treatment of a tumor-bearing animal with Hcy and L-methionase. Du Vigneaud et al. had shown very early (23) that Hcy can be utilized very well by normal animals and is not toxic. Apart from immunological problems and problems of access of tumor tissue to the enzymes, there are other critical obstacles to this type of therapy. On the one hand, as Kreis and Goodenow report (22), there are methionine-independent human tumor cell lines and, on the other hand, as observed by Hoffman et al. (8), at least some of the methionine-dependent cells revert at high frequency to methionine independence. These revertants would have a selective advantage under the conditions of methionase and Hcy infusions. It is true, however, that some of the revertants lose some of their properties associated with oncogenic transformation (18). Given these caveats this type of treatment seems promising, especially in light of the fact that Kreis has now isolated a new methionase from a Pseudomonas with a $K_a$ of 0.5 µM for methionine (Kreis, W., personal communication), and it should be initiated with tumor-bearing animals.

Conclusions and Prospects

Methionine dependence occurs in a large number and wide variety of cancer cells and does not seem to be a random component of the transformed phenotype although some tumor cells are methionine independent. However, Jacobsen et al. (25) indicate that at least one SV40-transformed human cell line, WI38VA13, has altered Met metabolism but is not completely methionine-dependent. Therefore, it is possible that altered Met metabolism may appear in various forms in cancer cells.

It thus remains to elucidate the basic biochemistry of methionine dependence. In this vein, it is very important to understand the relationship, if any, of methionine dependence to the cysteine dependence found in certain malignant cells (26-29) and to the adenosine deaminase deficiency seen in other malignant cells (30). Both cysteine and adenosine metabolisms, of course, are intimately related to Met metabolism (Fig. 2). In addition, it
has recently been shown that the betaine-dependent methionine synthetase (betaine: homocysteine methyltransferase, Fig. 2) can be induced in cultured cells (2). It is necessary to know what effect the induction of this enzyme will have on methionine dependence. It is knowledge of this basic biochemistry, which may involve critical cellular methylations, that may indicate the relationship of altered Met metabolism to oncogenic transformation and may reveal fundamental aspects of the mechanism of oncogenic transformation. This is especially so in light of new progress in isolation of oncogenes (31) and the fact that genes may be controlled by methylation (32).

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This work was supported by Grant CA27564 from the National Institutes of Health; The Council for Tobacco Research-USA, Inc.; The United Cancer Council, Inc.; The Cancer Research Coordinating Committee of the University of California; Grants from the Academic Senate, University of California, San Diego; and a Special Fellowship to R. M. H. from the Leukemia Society of America. We stand indebted to Professor Jerry A. Schneider for his generosity.