Vol. 117, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 16, 1983 Pages 429-434

THE METABOLIC DEFECT OF METHIONINE DEPENDENCE OCCURS FREQUENTLY IN HUMAN TUMOR CELL LINES

James O. Mecham, David Rowitch, C. Douglas Wallace, Peter H. Stern and Robert M. Hoffman*

Department of Pediatrics, M-009, University of California at San Diego School of Medicine, La Jolla, CA 92093

Received November 1, 1983

Methionine dependence is the inability of cells to grow when methionine (Met) is replaced by its immediate precursor homocysteine (Hcy) in the culture medium (Met^-Hcy^+ medium). All normal unestablished cell strains tested to date have been shown to be methionine-independent and thus grow almost as well in Met^-Hcy^+ medium as they do in Met^+Hcy^- medium. Results presented here indicate that out of 23 cell lines derived from diverse types of human tumors, 11 do not grow at all in Met^-Hcy^+ medium and are absolutely methionine-dependent and 3 grow only slightly in this medium. Many of the tumor cell lines tested have little else in common other than the fact that they are methionine-dependent. The high frequency of occurrence of methionine dependence in diverse types of human tumor cells indicates that methionine dependence may be an important aspect of oncogenic transformation and therapeutically exploitable.

The molecular basis of oncogenic transformation is still not understood. Metabolic characteristics common to diverse types of cancer cells but not to normal cells may shed light on the process of oncogenic transformation.

A potential candidate for such a metabolic characteristic is the defect of methionine dependence previously seen in some SV40-transformed human cells, a few human tumor cell lines and in some animal tumor cell lines. See Reference 1 for review. The defect is the inability of the cells to grow in medium in which methionine is replaced by its immediate precursor homocysteine (Met^-Hcy^+ medium). Normal unestablished cell strains thus far characterized grow well in Met^-Hcy^+ medium (1,2). If methionine dependence is important in human cancer, it should be prevalent in a large number of diverse types of human cancer cells from various sites. A survey of such cells for methionine dependence is the subject of this report.

*To whom all correspondance should be addressed.

0006-291X/83 $1.50

MATERIALS AND METHODS

The human tumor cell lines used in this survey and their origin are listed in Table 1. All except the following cells were grown in Eagle's MEM with 10% fetal bovine serum: A498, 8387, HT1080, A204, A2182, J82 and SK-N-SH, which were grown in Dulbecco's Modified MEM with 15% fetal bovine serum and T24, which was grown in McCoy's 5A with 20% serum. All media were supplemented with 100μM folic acid, 1.5μM hydroxocobalamin and either 100μM L-methionine or 200μM D,L-homocysteine thiolactone in addition to 0.1 mg/ml gentamycin-HCl. The fetal bovine serum was dialyzed against three changes of physiological saline before use. Cells were grown in 35mm petri dishes, detached with trypsin and enumerated in a Coulter Counter. Each data point is the result of three independent cell cultures. In all cases the growth of the replica cultures was essentially indistinguishable.

RESULTS

Although a number of human fibroblast cell strains have been tested and all shown to be methionine-independent (1), for comparative purposes we have tested three additional: all derived from human foreskin. As can be seen from Figure 1, these fibroblasts grow almost as well in $Met^{-}Hcy^{+}$ medium as in $Met^{+}Hcy^{-}$ medium and are therefore methionine-independent.

The growth behavior of the human tumor-derived cell lines, however, can be classified into 3 basic categories:

A) Those cells that are absolutely methionine-dependent and do not grow in $Met^{-}Hcy^{+}$ medium.

B) Those cells that are relatively methionine-dependent and whose growth is greatly reduced in $Met^{-}Hcy^{+}$ medium as compared to $Met^{+}Hcy^{-}$ medium.

C) Those cells that are methionine-independent and grow essentially equally well in both types of media.

Of the 23 tumor cells surveyed in this study, eleven fit into category A of being totally methionine-dependent and three fit into category B of being almost methionine-dependent. This leaves only nine of the twenty-three cell lines basically unaffected by substituting homocysteine for methionine in the culture media (Figure 1, Table 1)

Figure 1 groups the tumor cell lines by their origin and growth behavior. Among the carcinomas, two lung, one breast, one kidney, one prostate and one bladder carcinoma are absolutely methionine-dependent. However, two lung, one cervical, one colon, one prostate and one bladder carcinoma do show some growth in $Met^{-}Hcy^{+}$ medium although the growth of the cervical and colon

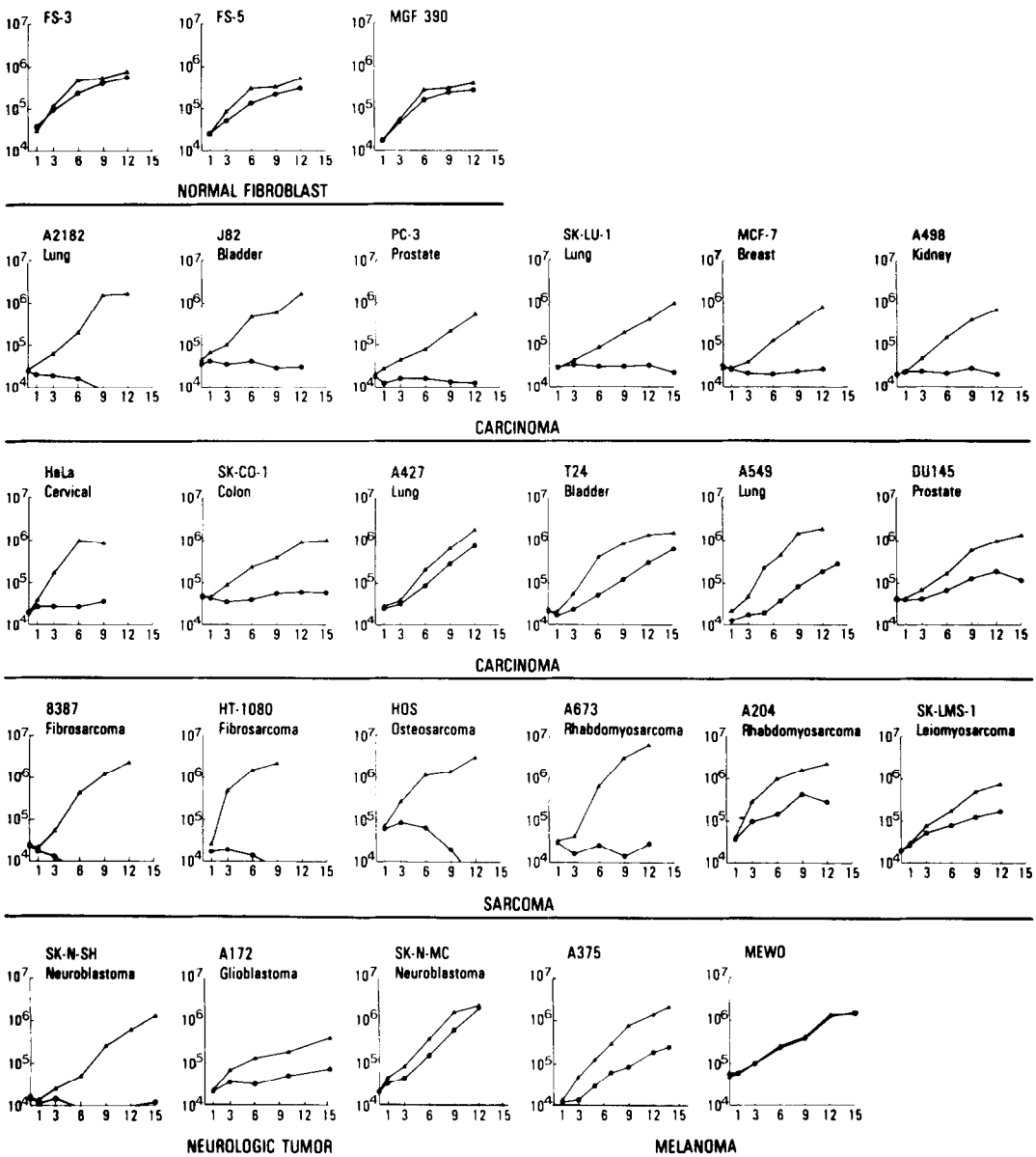

Fig. 1 Growth of human normal and tumor-derived cell lines in methionine-containing, homocysteine-depleted ($Met^{+}Hcy^{-}$) medium (▲-▲-▲) and in methionine-depleted, homocysteine-containing ($Met^{-}Hcy^{+}$) medium (●-●-●) . The Y-axis for each graph represents cell number per 35mm culture dish. The X-axis represents time in days. Each data point represents 3 independent determinations. See Materials and Methods for details.

carcinomas is very slight. Of the sarcomas, two fibrosarcomas, one rhabdomyosarcoma and an osteogenic sarcoma are absolutely methionine-dependent. In contrast, a leiomyosarcoma and another rhabdomyosarcoma grow almost as well in $Met^{-}Hcy^{+}$ medium as they do in

TABLE I. HUMAN CELL LINES USED IN STUDY

CELL LINE	ORIGIN	REFERENCE	DEGREE OF METHIONINE DEPENDENCE
FS-3	Normal human foreskin fibroblast	4	Independent
FS-5	Normal human foreskin fibroblast	4	Independent
MGF390	Normal human foreskin fibroblast	7	Independent
A2182	Lung carcinoma	8	Absolutely dependent
J82	Primary transitional cell carcinoma of bladder	9	Absolutely dependent
PC3	Prostate adenocarcinoma - metastasis to bone marrow	10	Absolutely dependent
SK-LU	Adenocarcinoma of lung	9	Absolutely dependent
MCF-7	Adenocarcinoma of breast - pleural effusion	11	Absolutely dependent
A498	Carcinoma of kidney	12	Absolutely dependent
HeLa	Cervical carcinoma	13	Slightly independent
SK-CO-1	Adenocarcinoma of colon	14	Slightly independent
A427	Carcinoma of lung	12	Independent
T24	Primary transitional cell carcinoma of bladder	15	Independent
A549	Adenocarcinoma of lung	12	Independent
DU145	Carcinoma of prostate - metastasis to brain	16	Independent
8387	Fibrosarcoma	17	Absolutely dependent
HT1080	Fibrosarcoma	18	Absolutely dependent
HOS	Osteogenic sarcoma	19	Absolutely dependent
A673	Rhabdomyosarcoma	12	Absolutely dependent
A204	Rhabdomyosarcoma	20	Independent
SK-LMS	Primary vulva leiomyosarcoma	20	Independent
SK-N-SH	Neuroblastoma - metastasis to bone marrow	21	Absolutely dependent
A172	Glioblastoma	12	Slightly independent
SK-N-MC	Neuroblastoma - metastasis to supraorbital area	22	Independent
A375	Malignant melanoma	12	Independent
MeWo	Malignant melanoma - metastasis to lymph node	23	Independent

$Met^{+}Hcy^{-}$ medium. With regard to cell lines which are derived from cancers of the nervous system, one neuroblastoma is absolutely methionine-dependent, while another neuroblastoma is completely methionine-independent and a glioblastoma is almost methionine-dependent. Of the two melanomas tested, one appears to be almost methionine-independent and the other is completely methionine-independent.

In summary, the data in Figure 1 indicate that the metabolic defect of methionine dependence, while not observed in normal unestablished cell strains, is highly prevalent among tumor cell lines diverse in their tissue type and site of origin.

DISCUSSION

As mentioned in the introduction, a metabolic charateristic or defect present in cancer cells but not in normals may shed light on the molecular basis of oncogenic transformation. The defect of methionine dependence, although not universally present in cancer cell lines, is present in tumor cell lines that seem to have little else in common other than oncogenic transformation. In contrast, all normal unestablished cell strains measured, including fibroblasts (Figure 1) and reference (1) and human epithelial cells (2), are methionine-independent.Indeed, the whole animal is methionine independent and grows well when homocysteine, vitamin B_{12} and folic acid replace methionine in the diet (24). It should be noted that two groups (25,26) have claimed that cell types they call "normal" are methionione dependent. However, these various cell types are actually immortalized cell lines, which precludes these cells being normal, and indeed immortalization is the first step toward oncogenic transformation (27-29). Additionally these authors (25,26) used concentrations of folic acid and vitamin B_{12} in their media insufficient for optimal growth in homocysteine(30). Thus, methionine dependence may have a relationship to oncogenic transformation, and understanding the biochemistry of the former (3-6) should contribute to our understanding of the biochemistry of the latter.

ACKNOWLEDGEMENTS. This study was supported by grants 1348 and 1496 from the Council for Tobacco Research-USA, Inc., NIH Research Career Development Award CA00804, NIH/National Cancer Institute grant CA27564 and the George A. Jacobs Memorial Fund for Cancer Research, all to Robert M. Hoffman. James O. Mecham's work was supported by NIH training grant AM07318.

REFERENCES

1. Hoffman, R.M. (1982) In Vitro 18, 421-428.
2. Lechner, J. Personal communication.

3. Coalson, D.W., Mecham, J.O., Stern, P.H. and Hoffman, R.M. (1982). Proc. Natl. Acad. Sci. 79, 4248-4251.
4. Stern, P.H., Mecham, J.O., Wallace, C.D. and Hoffman, R.M. (1983) J. Cellular Physiology 117, 9-14.
5. Hoffman, R.M. and Erbe, R.W. (1976) Proc. Natl. Acad. Sci. 73, 1523-1527.
6. Hoffman, R.M. and Jacobsen, S.J. (1980) Proc. Natl. Acad. Sci. 77, 7306-7310.
7. Jacobsen, S.J., Hoffman, R.M. and Erbe, R.W. (1980) J. Natl. Canc. Inst. 65, 1237-1244.
8. Pulciani, S., Santos, E., Lauver, A.V., Long, S.K., Robbins, K.C. and Barbacid, M. (1981). Proc. Natl. Acad. Sci. USA 79, 2845-2849.
9. Fogh, J. (1978) Natl. Cancer Inst. Monogr. 49, 5-9.
10. Kaighn, M., Narayan, K.S., Ohnuki, Y., Lechner, J. and Jones, L.W. (1979). Invest. Urol. 17, 16-23.
11. Soule, H.D., Vazquez, J., Long, A., Albert, S., Brennan, M. (1973). J. Natl. Cancer Inst. 51, 1409-1416.
12. Giard, D.J., Aaronson, S.A., Todaro, G.J. (1973). J. Natl. Canc. Inst. 51, 1417-1423.
13. Diala, E.S. and Hoffman, R.M. (1982). Biochem. Biophys. Res. Commun. 107, 19-26.
14. Fogh, J. and Trempe, G. (1975) In Fogh, J., Ed., Human Tumor Cell Lines in Vitro, New York, Plenum Press, pp. 115-119.
15. Bubenik, J., Baresova, M., Vinlicky, V., Jakoubkova, J., Sainerova, H. and Donner, J. (1973). Int. J. Cancer 11, 767-773.
16. Mickey, D., Stone, K.R., Wunderli, , Mickey, G.H., Vollmer, R.T. and Paulson, D.F. (1977). Cancer Res. 37, 4049-4058.
17. Aaronson, S.A., Todaro, G.J. and Freeman, A.E. (1970) Exp. Cell Res. 61, 1-5.
18. Rasheed, S., Nelson-Rees, W.A., Toth, E.M. et.al. (1974). Cancer 33, 1027-1033.
19. Eva, A., Robbins, K.C., Anderson, P.R. et.al. (1982) Nature 295, 116-119.
20. Fogh, J., Wright, W. and Loveless, J. (1982) Nature 295, 116-119.
21. Biedler, J.L., Helson, L. and Spengler, B.A. (1973) Cancer Res. 33, 2643-2652.
22. Spengler, B., Biedler, J.L., Helson, L. and Friedman, L.S. (1973). In Vitro 8, 410.
23. Carey, T.E., Takahashi, T., Resnick, L.A., Oettgen, H.F. and Old, L.J. (1976). Proc. Natl. Acad. Sci. USA 73, 3278-3282.
24. du Vigneaud, V., Ressler, C. and Rachele, J. (1950). Science 112, 267-271.
25. Kano,Y., Sakamoto,S., Kasahara, T., Kusumoto, K., Hida, K., Suda, K., Ozawa, K., Miura, Y. and Takaku, F. (1982). Cancer Res. 42, 3090-3092.
26. Carson, D., Willis, E. and Kamatani, N. (1983) Biochem. Biophys. Res. Commun. 112, 391-397.
27. Land. H., Parada, L. and Weinberg, R.A. (1983) Nature 304, 596-602.
28. Ruley, H.E. (1983). Nature 304, 602-606.
29. Newbold, R. and Overell, R. (1983). Nature 304, 648-651.
30. Taylor, R.T. and Hanna, M.L. (1975) Archives of Biochemistry and Biophysics 171, 507-520.