

# Methionine Dependence of Cancer

Subjects: **Biology**

Contributor: Peter Kaiser

Tumorigenesis is accompanied by the reprogramming of cellular metabolism. The shift from oxidative phosphorylation to predominantly glycolytic pathways to support rapid growth is well known and is often referred to as the Warburg effect. However, other metabolic changes and acquired needs that distinguish cancer cells from normal cells have also been discovered. The dependence of cancer cells on exogenous methionine is one of them and is known as methionine dependence or the Hoffman effect. This phenomenon describes the inability of cancer cells to proliferate when methionine is replaced with its metabolic precursor, homocysteine, while proliferation of non-tumor cells is unaffected by these conditions. Surprisingly, cancer cells can readily synthesize methionine from homocysteine, so their dependency on exogenous methionine reflects a general need for altered metabolic flux through pathways linked to methionine.

methionine

S-adenosylmethionine

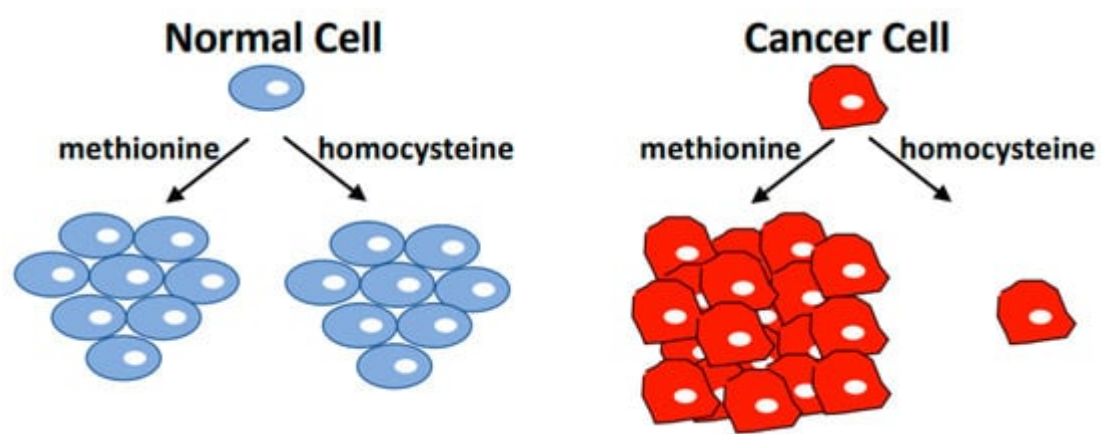
cell cycle

cancer

SAM-checkpoint

## 1. Introduction

In 1959, Sugimura and colleagues reported a study where tumor-bearing rats were fed diets with the restriction of individual essential amino acids. Tumor growth was significantly affected by a methionine-restricted diet <sup>[1]</sup>. The dependence of cancer cell proliferation on methionine was further highlighted in 1973 by experiments that showed leukemia cells cannot proliferate in growth media where methionine is substituted with its metabolic precursor, homocysteine <sup>[2]</sup>. A flurry of experiments during the 70s and 80s expanded the methionine/homocysteine substitution experiments to many cell lines derived from various tumor sites. The results unequivocally established that the vast majority of cancer cells cannot proliferate when methionine is replaced with homocysteine, but non-cancer cells are indifferent to such replacement <sup>[3][4][5][6][7]</sup>. [Table 1](#) lists cell lines with a known status of methionine dependence. This metabolic phenomenon that differentiates cancer cells from non-tumor cells is often referred to as the methionine dependence of cancer, the methionine stress sensitivity of cancer, or the Hoffman effect ([Figure 1](#)). It soon became clear that the addiction of cancer cells to exogenously provided methionine is not due to their failure to synthesize methionine from homocysteine <sup>[3][8]</sup>, but is likely caused by increased demand for metabolites derived from methionine <sup>[5][7][8][9]</sup>. While mechanisms behind the Hoffman effect are yet to be fully understood, progress has been made towards this goal.



**Figure 1.** The Hoffman Effect. Non-tumorigenic cell lines have the same proliferation rate in media containing methionine or media where methionine is replaced with the immediate metabolic precursor homocysteine. However, most cancer cells cannot proliferate in homocysteine medium, and induce cell cycle arrest followed by apoptosis when cultured under these conditions. Cancer cells readily synthesize methionine from homocysteine, but appear to depend on exogenously supplied methionine. This cancer-specific metabolic dependence is referred to as the methionine dependence of cancer, the methionine stress sensitivity of cancer, or simply the Hoffman effect.

**Table 1.** Cell lines with known growth properties in homocysteine medium.

Cell Line	Methionine Dependence	Tumor Site
MDA-MB468	Yes [7][10]	Breast
MCF7	Yes [5][6][7][10]	Breast
MDA-MB361	Yes [7]	Breast
HCC1806	Yes [10]	Breast
HCC1143	Yes [10]	Breast
SKBR3	Yes [10]	Breast
BT-549	Yes [10]	Breast
ZR-75-1	Yes [10]	Breast
SUM-159	Yes [10]	Breast
T47D	Yes [10]	Breast
W-256	Yes [3][4]	Breast (rat)
MDA-MB231	No [1], moderate [10]	Breast

Cell Line	Methionine Dependence	Tumor Site
HCC70	No <a href="#">[10]</a>	Breast
HCC38	No <a href="#">[10]</a>	Breast
SUM-149	No <a href="#">[10]</a>	Breast
MDA-MB231	No <a href="#">[1]</a> , moderate <a href="#">[10]</a>	Breast
BxPC3	Yes <a href="#">[11]</a>	Pancreas
PANC1	No <a href="#">[11]</a>	Pancreas
LoVo	Yes <a href="#">[12]</a>	Colon
SK-CO-1	Yes <a href="#">[6]</a>	Colon
PC-3	Yes <a href="#">[5]</a> <a href="#">[6]</a> <a href="#">[13]</a>	Prostate
LNCaP	Moderate <a href="#">[13]</a>	Prostate
DU145	Moderate <a href="#">[5]</a> <a href="#">[6]</a> <a href="#">[13]</a>	Prostate
SV80	Yes <a href="#">[3]</a>	Transformed fibroblast
HEK293T	Yes <a href="#">[11]</a>	Transformed kidney cell
W18VA2	Yes <a href="#">[3]</a>	SV40 transformed human cells
J111	Yes <a href="#">[4]</a>	Monocytic leukemia
L1210	Yes <a href="#">[4]</a>	Lymphatic leukemia (mouse)
A2182	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Lung
SK-LU-1	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Lung
A549	Moderate <a href="#">[6]</a>	Lung
A427	No <a href="#">[5]</a> <a href="#">[6]</a>	Lung
J82	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Bladder
T24	No <a href="#">[5]</a> <a href="#">[6]</a>	Bladder
8387	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Fibrosarcoma
HT1080	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Fibrosarcoma
HOS	Yes <a href="#">[5]</a> <a href="#">[6]</a>	Osteosarcoma

Cell Line	Methionine Dependence	Tumor Site
A204	Moderate <sup>[6]</sup>	Rhabdomyosarcoma
A673	Yes <sup>[5][6]</sup>	Rhabdomyosarcoma
SK-LMS1	No <sup>[5][6]</sup>	Leiomyosarcoma
SK-N-SH	Yes <sup>[5][6]</sup>	Neuroblastoma
SK-N-MC	No <sup>[6]</sup>	Neuroblastoma
A375	Moderate <sup>[6]</sup>	Melanoma
MeWo	No <sup>[6]</sup>	Melanoma
A172	Moderate <sup>[5][6]</sup>	Glioblastoma
HeLa	Yes <sup>[6]</sup>	Cervical
A498	Yes <sup>[5][6]</sup>	Kidney

1. Chene, P.H.; Stern, P.H.; Hoffman, R.M. Dependence of methylating agent sensitivity of L1210 murine leukemia cells in vitro on the presence of hydroxycobalamin and transcobalamin II. Cancer Res. 1973, 33, 1898–1904.

## 2. Methionine Metabolism

2. Hoffman, R.M.; Stern, P.H.; Chene, P.H. Regulation of rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc. Natl. Acad. Sci. USA 1976, 73, 1523–1527.

Methionine is an essential amino acid in mammals. In addition to its role as a component of proteins, methionine links to a number of important metabolic pathways that play key roles in epigenetics (S-adenosylmethionine), nuclear functions (polyamines), detoxification (glutathione), and cellular membranes (phospholipids) (Figure 2).

4. Halpern, B.C.; Clark, B.R.; Hardy, D.N.; Halpern, R.M.; Smith, R.A. The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture. Proc. Natl. Acad. Sci. USA 1974, 71, 1133–1136.

5. Stern, P.H.; Wallace, C.D.; Hoffman, R.M. Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines. J. Cell. Physiol. 1984, 119, 29–34.

6. Mecham, J.O.; Rowitch, D.; Wallace, C.D.; Stern, P.H.; Hoffman, R.M. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem. Biophys. Res. Commun. 1983, 117, 429–434.

7. Booher, K.; Lin, D.W.; Borrego, S.L.; Kaiser, P. Downregulation of Cdc6 and pre-replication complexes in response to methionine stress in breast cancer cells. Cell Cycle 2012, 11, 4414–4423.

8. Borrego, S.L.; Faltmann, J.; Datta, R.; Stringari, C.; Grapov, D.; Zeller, M.; Chen, Y.; Wang, P.; Baldi, P.; Gratton, E.; et al. Metabolic changes associated with methionine stress sensitivity in MDA-MB-468 breast cancer cells. Cancer Metab. 2016, 4, 9.

**Figure 2.** Methionine metabolism. (A) Metabolic connections between the methionine cycle, which produces S-adenosylmethionine, the methionine salvage pathway, which recycles methionine from byproducts of the polyamine synthesis pathway, and the transsulfuration pathway, which generates cysteine and glutathione to

10. Lin, D.-W.; Geller, A.M.; Epner, D.E.; Kaiser, P.; University of California, Irvine. Unpublished work. 2020. Methionine dependence of cancer cells through the cystine-glutamate antiporter xCT. *Sci. Signal.* 2017, 10, pii:eaa06604.

Methionine is obtained through the diet. It is converted to the principal cellular methyl donor, S-adenosylmethionine (SAM, also referred to as AdoMet), through the transfer of adenosine from ATP to the methionine sulfur. This

11. Sun, Y.; Kaiser, P.; University of California, Irvine. Unpublished work. 2020. transferase in the liver,

whereas extrahepatic tissues rely on the Mat2A/Mat2B complex for SAM synthesis. Mat2A is the catalytic subunit, but binding of the regulatory subunit Mat2B modifies kinetic properties by decreasing the  $K_m$  for methionine and

12. Lu, S.; Epner, D.E. Molecular mechanisms of cell cycle block by methionine restriction in human

prostate cancer cells. *Nutr. Cancer* 2000, 38, 123–130. product inhibition [14][15][16]. SAM is used as a cofactor in most methylation reactions and

13. Koth, M.; Kredich, N.M. Regulation of human lymphocyte S-adenosylmethionine synthetase by

product inhibition. *Biochim. Biophys. Acta* 1990, 1039, 253–260. remains after the methyl group transfer and is hydrolyzed into homocysteine and adenosine by SAH hydrolase.

14. Hahn, A.-B.B.; LeGros, L.; Geller, A.; Koth, M. Expression and functional interaction of the

homocysteine is then remethylated using 5-methyltetrahydrofolate as the methyl group donor to regenerate

15. Hahn, A.-B.B.; LeGros, L.; Geller, A.; Koth, M. Expression and functional interaction of the

16. LeGros, H.L.; Geller, A.M.; Koth, M. Differential Regulation of Methionine Adenosyltransferase in

do not express the necessary enzyme, betaine-homocysteine methyltransferase [17][18], and rely on the folate and

17. Uhlen, M.; Fagerberg, L.; Hallström, B.M.; Lindskog, C.; Oksvold, P.; Mardinoglu, A.; Sivertsson,

A.; Kampf, C.; Sjostedt, E.; Asplund, A.; et al. Tissue-based map of the human proteome. *Science*

2015, 347, 1260419. ability of cells to methylate substrates is thus not only determined by SAM abundance, but also by SAH levels. The

cellular methylation potential is thus best expressed as the SAM/SAH ratio.

18. The Human Protein Atlas. Available online: (accessed on 8 March 2020).

19. Coward, J.K.; Slisz, E.P. Analogs of S-adenosylhomocysteine as potential inhibitors of biological

transmethylation. Specificity of the S-adenosylhomocysteine binding site. *J. Med. Chem.* 1973,

16, 460–463. Methionine metabolism has been connected to cancer on several levels. This review focuses on the Hoffman

effect, which describes the dependence of cancer cells on exogenous methionine. Most cancer cells cannot

20. Hoffman, R.M.; Jacobsen, S.J.; Erbe, R.W. Reversion to methionine independence by malignant

proliferate in medium where methionine is replaced by homocysteine, even though they readily synthesize

methionine from homocysteine (Figure 1). In fact, when intracellular methionine levels were measured in breast

21. Hoffman, R.M.; Jacobsen, S.J.; Erbe, R.W. Reversion to methionine independence in simian virus

40-transformed human and malignant rat fibroblasts is associated with altered ploidy and altered

properties of transformation. *Proc. Natl. Acad. Sci. USA* 1979, 76, 1313–1317. and normal cells can indirectly lead to distinct metabolic needs. However, the existence of several rapidly

proliferating methionine-independent cancer cell lines argues against a major influence of proliferation rate (Table

22. Borrego, S.L.; Lin, D.-W.; Kaiser, P. Isolation and Characterization of Methionine-Independent

1) [5][6]. Why some cancer cells remain methionine independent is not well understood. In addition, more direct

approaches have definitely excluded indirect effects from growth rate-related metabolic dependence [7][8][20][21][22].

23. Vanhamme, L.; Szpirer, C. Spontaneous and 5-azacytidine-induced revertants of methionine-

dependent tumor-derived and H-ras-1-transformed cells. *Exp. Cell Res.* 1989, 181, 159–168. Most notably, when methionine-dependent cancer cells are continuously cultured in homocysteine medium, very

rare cell clones can be selected that reverted to methionine independence without a change in proliferation rate [7]

[8][20][21][22]. Remarkably, most of these cancer cell-derived methionine-independent clones have lost properties

associated with the tumorigenic state [8][20]. The mechanism of reversion is not known, but chromosomal alterations



24. Tisdale, M.R. Utilization of preformed and endogenously synthesized methionine by cells in tissue of culture. *Mol. Cell Biochem.* 1984; 49, 315–320.

MDA-MB468 Breast Cancer 1984; 49, 315–320 seems to be an epigenetic event, because the reverted state is semi-stable and needs to be stabilized occasionally by growth in homocysteine medium [22]. As indicated above, generation of methionine stress resistant cancer cells is usually coupled with loss of tumorigenic properties such as Homocysteine and Cysteine. *Annu. Rev. Nutr.* 2004, 24, 539–577.

25. Stipanuk, M.H. SULFUR AMINO ACID METABOLISM: Pathways for Production and Removal of Homocysteine and Cysteine. *Annu. Rev. Nutr.* 2004, 24, 539–577.

26. Mehrmohamadi, M.; Liu, X.; Shestov, A.A.; Locasale, J.W. Characterization of the Usage of the Phosphoinositide 3-Kinase (PI3K) and R-Ras expression have been shown to promote methionine dependence [23]. Serine Metabolic Network in Human Cancer. *Cell Rep.* 2014, 9, 1507–1519.

27. Verschoor, M.L.; Singh, G. Ets-1 regulates intracellular glutathione levels: Key target for resistant ovarian cancer. *Mol. Cancer* 2013, 12, 138.

28. Borrego, S.L.; Kaiser, P.; University of California, Irvine. Unpublished work, 2018.

29. Maddocks, O.D.K.; Labuschagne, C.F.; Adams, P.D.; Vousden, K.H. Serine Metabolism Supports the Methionine Cycle and DNA/RNA Methylation through De Novo ATP Synthesis in Cancer Cells. *Mol. Cell* 2016, 61, 210–221.

30. Mattaini, K.R.; Sullivan, M.R.; Vander Heiden, M.G. The importance of serine metabolism in cancer. *J. Cell Biol.* 2016, 214, 249–257.

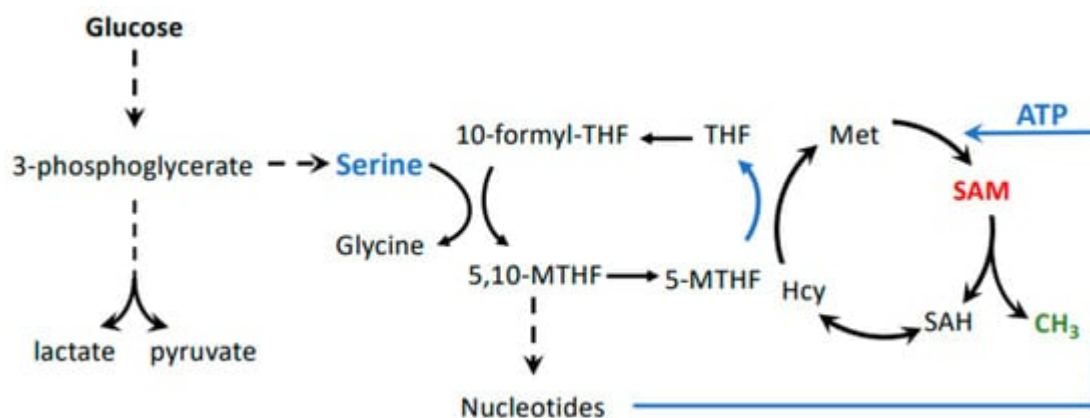
31. Labuschagne, C.F.; van den Broek, M.J.F.; Mackay, C.M.; Vousden, K.H.; Maddocks, O.D.K. Serine, but Not Cysteine, Supports One-Carbon Metabolism and Proliferation of Cancer Cells. *Cell Rep.* 2014, 7, 1243–1258.

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For example, methionine levels may be kept constant at the expense of flux into downstream pathways. Indeed, tracing experiments in MDA-MB468 breast cancer cells with labelled homocysteine revealed a diversion of flux from the methionine cycle to the transsulfuration pathway (Figure 2A) [8]. These same experiments indicated the reduced synthesis of SAM, resulting in a reduced overall methylation potential reflected in a lower SAM/SAH ratio. These tracing experiments suggested that low SAM or SAM/SAH ratios are key to understanding the Hoffman effect, and that increased flux through the transsulfuration pathway may induce these changes in methylation potential. Notably, none of these metabolic effects were observed in revertant, methionine-independent MDA-MB468-R8 clones. The differential metabolic fates of homocysteine in methionine-dependent (transsulfuration) and -independent cells (mainly methionine cycle) observed in breast cancer cell systems may be a general feature of the Hoffman effect. Indeed, similar redirection of homocysteine into the transsulfuration branch is induced by oncogenic PI3K mutations, which induce a methionine-dependent phenotype when expressed in methionine-independent cells (Figure 2A) [10]. It is interesting to note that the transsulfuration pathway is largely restricted to pancreas, liver, and kidney, but is active in cancer cells [8][25][26][27], which could contribute to the differential response of cancer cells to growth in homocysteine medium. One can speculate that higher demand for antioxidants like glutathione in cancer may require increased flux through the transsulfuration pathway. However, at least in MDA-MB468 breast cancer cells, supplementation with antioxidants cannot compensate for methionine dependence [28].

Effects of methionine substitution with homocysteine on cellular methylation potential have been observed previously [3], but the significance of SAM as an important mediator of the Hoffman effect became clear when SAM supplementation of homocysteine medium eliminated the growth defect of MDA-MB468 cells [7]. Accordingly,

reducing SAM synthesis directly by knockdown of MAT2A/B without affecting methionine levels resulted in a similar cell cycle arrest as observed by methionine replacement [9]. The realization that SAM is one of the key metabolites related to the methionine dependence of cancer may also connect other metabolic pathways important to cancer with the Hoffman effect. For example, the serine–glycine biosynthesis pathway is critical to maintaining cellular methylation potential (Figure 2B). This pathway maintains flux through the folate cycle. Thereby, serine to glycine conversion sustains homocysteine remethylation to methionine, but also stimulates nucleotide biosynthesis, including ATP, which, together with methionine, provides the building blocks for SAM (Figure 3) [29]. Several cancer cell lines and tumors depend on exogenous serine. This is surprising because serine can usually be synthesized from glucose in sufficient amounts via the glycolysis intermediate 3-phosphoglycerate [30][31]. However, the increased demand of cancer cells on glycolysis for energy production may divert the flux of 3-phosphoglycerate from serine synthesis to glycolysis. Serine requirement for cancer cell proliferation may thus link the Warburg and Hoffman effects by connecting glycolytic flux with SAM synthesis.



**Figure 3.** Possible connection between the Warburg and Hoffman effects. Glycolysis is connected to the methionine cycle through the folate cycle, whereby serine derived from 3-phosphoglycerate provides one-carbon units. This pathway is important in cancer cells because the folate cycle feeds both nucleotide and SAM biosynthesis. SAM levels are especially impacted by the folate cycle because it is important for re-methylation of homocysteine to methionine, as well as ATP synthesis. Both methionine and ATP are substrates for formation of SAM.