

Human Tumors Are Methionine Dependent *in Vivo*

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Abstract. Methionine-dependence is a tumor-specific biochemical defect expressed by the inability or decreased ability of tumors to grow under the condition of methionine-depletion. Many reports have shown that methionine-dependence occurs in human tumors of all types, including fresh surgical specimens *in vitro*. However, *in vivo* determinations of methionine-dependence have thus far been made only in rodent malignant tumors using methionine-deficient diets. We report here for the first time that human cancer xenografts in nude mice are methionine-dependent and when fed a methionine-free diet tumor growth is greatly inhibited. The body weight of mice on the methionine-free diet was found to be maintainable by once-per-week administration of methionine. The data presented here suggest that methionine-dependence can be an important target for human cancer treatment.

Methionine-dependence is the inability of tumor cells to grow under the condition of methionine-depletion. Methionine-dependence has been observed in malignant tumors, but not in normal cells (1). Methionine dependence is probably due at least in part to excess methionine-utilization for methylation reactions in tumor cells (2).

Under the condition of methionine-depletion, methionine-dependent cells, unlike normal cells, cannot divide and arrest in the late-S/G₂ phase of the cell cycle (3, 4). The selective cell cycle arrest of tumor cells by methionine-depletion allows enhanced efficacy of cell-cycle specific drugs against the tumor cells (5). The tumor-selective late-S/G₂ arrest due to methionine-depletion has been observed *in vitro* (3) and *in vivo* (4). We have reported that many human malignant cell lines and fresh human cancers originating from all organs were methionine-

dependent *in vitro* (6, 7). Rodent tumors, in particular the Yoshida sarcoma, have been found to be methionine-dependent *in vivo* (7, 8, 9, 10). However, no studies have been done as yet to determine whether human tumors are methionine-dependent *in vivo* and can thereby be growth-inhibited by restricting their methionine source. We report here for the first time that methionine-dependence actually occurs in human cancer *in vivo* in a study of xenografts using nude mice fed a methionine-free diet.

Materials and Methods

Mice. Six-week-old male and female BALB/C nu/nu mice, originating from the Central Institute for Experimental Animals (Kawasaki, Japan), were purchased from CLEA, Tokyo, Japan, or bred at AntiCancer Inc. under NIH guidelines.

Tumor. Breast (R-27), two colon (Co-4, COLO-205), and two lung (H-460, H-522) cancer xenografts were used. R-27 was established as a cultured cell line from a tamoxifen-resistant variant of MCF-7 in 1981 by Nawata (11) and was successfully transplanted into nude mice in Keio University (12, 13). Co-4 was established in the Pathology Division of the Japanese National Cancer Center Research Institute. COLO-205, H-460 and H-522 were kindly supplied by Dr. Jacqueline Plowman of the U.S. National Cancer Institute. All the xenografts were maintained by serial transplantation in nude mice.

Transplantation. Tumors in the exponential growth phase in nude mice were resected aseptically, necrotic tissues were cut away, and the remaining viable tumor tissue was minced into pieces about 3 to 4 mm in diameter in Hanks' balanced salt solution. The pieces were transplanted to either side of the backs of nude mice subcutaneously. The female mice transplanted with the R-27 human breast cancer xenograft were administered with a one-time dose of 17- β -estradiol dipropionate at 5 mg/kg intramuscularly.

Diets. The defined diets, TD 930030 (methionine-containing diet) and TD 92077 (methionine-free diet), were purchased from TEKLAD (Madison, WI). Normal cells can synthesize methionine from homocysteine. Therefore the methionine-free diet was also depleted of homocysteine and choline to allow extensive depletion of methionine in the mouse blood. Mice were divided into two groups of four to five for transplantation. Mice in the control groups were fed the methionine-containing diet every day. Mice in the treatment groups were fed the methionine-free diet every day except for the mice in the treatment group bearing human

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Key Words: Methionine-dependence, human tumors, *in vivo*.

Table I. Effect of dietary methionine depletion on the growth of human tumor xenografts in nude mice.

| Human tumor xenograft | T/C of tumor weight(%) | P-value |
|-----------------------|------------------------|-------------|
| H-522 | 31.4 | 0.001 |
| COLO-205 | 33.6 | 0.041 |
| R-27 | 43.6 | 0.067(N.S.) |
| H-460 | 55.0 | 0.004 |
| Co-4 | 55.1 | 0.005 |

Tumors were transplanted subcutaneously and measured as described in the text. Tumor weights were compared at the end of the experiment in mice on the methionine-containing and methionine-free diets. Tumors were grown for 14 to 39 days.

breast tumor R-27, which were fed with the methionine-free diet for six consecutive days each week and with the methionine-containing diet one day each week.

Tumor measurement. The length and width of the tumors were measured with sliding calipers 3 times a week. The body weight of the mice was measured at the same time. The tumor weight was estimated according to the following formula.

$$\text{Tumor weight (mg)} = \text{Length (mm)} \times [\text{Width (mm)}]^2 / 2$$

If the mice showed signs of distress, they were sacrificed and the tumors were resected to measure the actual tumor weight.

Statistical analysis: The antitumor effect of the methionine-free diet was evaluated by the T/C ratio (%), where T was the actual tumor weight of the treated group and C was the actual tumor weight of the control group on sacrifice. Statistical analysis of the data was performed according to Student's-t test.

Results and Discussion

Table I shows the effect of the methionine-free diet on human breast cancer R-27, colon cancers Co-4 and COLO-205, and lung cancers H-460 and H-522. The growth of these tumors was inhibited in the mice fed the methionine-free diet with statistical significance for H-522, COLO-205, H-460, and Co-4.

The mice in the treatment group bearing R-27 were fed a methionine-containing diet one day a week to avoid excessive body weight loss. Even with the intermittent methionine-free diet, the T/C ratio was still 43.6% for this tumor. Lung cancer H-522 and colon cancer COLO-205 seemed to be the most methionine-dependent with T/C values of approximately 30%.

When the ratios of tumor inhibition to body weight loss due to the methionine-free diet were determined, it can be seen in Table II that the methionine-free diet had a strong selective effect against the tumors. Using this value, lung tumor H-522 and breast tumor R-27 were most methionine-dependent with ratios under 50%.

Indeed, for the R-27 tumor-bearing mice, the weekly schedule of 6 days on the methionine-deficient diet and one day on the methionine-containing diet reduced the body weight loss to only 10.2% over the 33-day treatment period.

The results presented here demonstrate that methionine-dependence actually occurs in human tumors *in vivo* and sug-

Table II. Effect of dietary methionine depletion on the ratio of tumor growth inhibition to body weight loss.

| Human tumor xenograft | T/C of tumor weight Relative body weight change |
|-----------------------|--|
| H-522 | 0.44 |
| R-27 | 0.49 |
| COLO-205 | 0.63 |
| H-460 | 0.79 |
| Co-4 | 0.79 |

Tumors were grown and measured in mice on the methionine-containing and methionine-free diets described in the text and Table I. Relative body weight change was the ratio of the body weight at the end to the body weight at the beginning of the methionine-free diet.

gest that methionine-dependence is a potential selective target for human cancer treatment.

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Received November 11, 1994

Accepted January 18, 1995