Methionine-dependence and combination chemotherapy on human gastric cancer cells in vitro

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INTRODUCTION

Gastric cancer is common in China and abroad[1-20], and chemotherapy is still a main method for advanced cancer so far[21-30]. Up to now, quite a few studies have elucidated the property of methionine (Met) dependence of cancer cells[31-34]. If that property was utilized in combination with specific chemotherapeutic drugs, the proliferation of tumor cells would be suppressed. This study intended to culture the human primary gastric cancer cells in diverse environments to judge whether the gastric cancer cells were Met-dependent and whether Met-Hcy+ could enhance the effect of chemotherapy.

MATERIALS AND METHODS

Study on Met-dependence

Human primary gastric cancer cells were collected from gastric cancer tissues of 37 advanced gastric cancer patients in our hospital, and human gastric mucosal epithelial cells were simultaneously obtained in 31 patients. Based on the Met-free RPMI-1640 medium, dialyzed bovine serum, and other ingredients necessary for cell growth, Met-free but homocysteine-containing (Met+Hcy-) medium, with other chemotherapeutic drugs, the number of surviving gastric cancer cells was used in combination with chemotherapeutic drugs, the number of surviving gastric cancer cells dropped significantly.

RESULTS: The growth of human primary gastric cancer cells in Met+Hcy-' was suppressed, manifestly by the decrease of total cell counts [1.46±0.42 (×10⁹·L⁻¹) in Met+Hcy- vs 1.64±0.44 (×10⁹·L⁻¹) in Met+Hcy', P<0.01], the decrease in the percentage of G2/G phase cells (0.69±0.24 in Met+Hcy' vs 0.80±0.18 in Met+Hcy', P<0.01) and the increase of S cells (0.24±0.20 in Met+Hcy' vs 0.17±0.16 in Met+Hcy', P<0.01); however, gastric mucosal cells grew normally. If Met+Hcy medium was used in combination with chemotherapeutic drugs, the number of surviving gastric cancer cells dropped significantly.

CONCLUSION: Human primary gastric cancer cells in vitro are Met-dependent; however, gastric mucosal cells have not shown the same characteristics. Met-Hcy' environment may strengthen the killing effect of chemotherapy on human primary gastric cancer cells.

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the similar phenomenon. The percentage of cells in G0G1 phases in the test group was significantly lower than that in the control group while the percentage of S phase cells in test group was obviously higher than that in the control group. There was no statistical significance between the percentage of G2M cells in the test group and that in the control group. The result of cell counting was that human primary gastric cancer cells in the test group were apparently less than those in the control group (Table 1).

**Table 1** The distribution of human primary gastric cancer cell cycle and the amount of cells (Tas, n = 37)

| Groups     | Fractions in different phases | Amount of cells (×10^6·L⁻¹) |
|------------|-------------------------------|-------------------------------|
|            | G0G1 | S     | G2M |                                         |
| Met-Hcy⁻   | 0.80±0.16 | 0.17±0.16 | 0.04±0.06 | 1.64±0.44 |
| Met-Hcy⁺   | 0.69±0.24 | 0.24±0.20 | 0.07±0.13 | 1.46±0.42 |

*P<0.01, vs Met-Hcy⁻ group.

**Human gastric mucosa cell cycle and amount**

Gastric mucosa epithelial cells grew well both in the test group and in the control group. There was no obvious difference in the percentages in various phases in cell cycle between the two groups. The amount of cells in the test group was slightly higher than that in the control group but without statistical significance (Table 2).

**Table 2** The distribution of human gastric mucosa epithelial cell cycle and the amount of cells (Tas, n = 31)

| Groups     | Fractions in different phases | Amount of cells (×10^6·L⁻¹) |
|------------|-------------------------------|-------------------------------|
|            | G0G1 | S     | G2M |                                         |
| Met-Hcy⁻   | 0.90±0.13 | 0.07±0.11 | 0.02±0.02 | 1.70±0.44 |
| Met-Hcy⁺   | 0.90±0.14 | 0.08±0.11 | 0.03±0.04 | 1.75±0.45 |

**Different media in combination with chemotherapeutic drugs**

The surviving gastric cancer cells in the test group without chemotherapeutic drugs were fewer than those in the corresponding control group, with lower A value. The proliferation of gastric cancer cells in the Met-Hcy⁻ group was suppressed much more strongly than that in the Met-Hcy⁺ group, no matter which drug was added. The former group had the manifestation of the decrease in surviving gastric cancer cells to various extents, with lower A value (Table 3).

**Table 3** The influence of Met-Hcy⁻ or Met-Hcy⁺ in combination with chemotherapeutic drugs to gastric cancer cells (Tas, A)

| Drug   | Met-Hcy⁻ | Met-Hcy⁺ |
|--------|-----------|----------|
| ADM    | 0.3807±0.3114 | 0.3175±0.2003 |
| DDP    | 0.3878±0.3050 | 0.3189±0.1848 |
| 5-FU   | 0.3657±0.2597 | 0.3182±0.2049 |
| MMC    | 0.3861±0.2734 | 0.3105±0.2103 |
| MTX    | 0.3649±0.2811 | 0.3120±0.2003 |
| Control| 0.4834±0.4337 | 0.3981±0.3056 |

*P<0.05, ^P<0.01, vs Met-Hcy⁻.

**DISCUSSION**

**Met-dependence and Met starvation**

Methionine is an essential amino acid containing an S-methyl, which is used to synthesize through methylation quite a few important physiological active substances. Homocysteine is the direct precursor of Met. Hcy and Met may be converted into each other in vivo, but it is not as such in vitro. Hcy is continuously consumed with the need of metabolism, and hence is transformed and supplied by Met. Met depletion in vivo is easily produced once Met supply is stopped, as there is no pathway in vivo of Hcy transforming to Met, which is an essential amino acid. Normal cells can grow well in the environment containing Hcy instead of Met. The proliferation of tumor cells, however, may be suppressed under such condition. This phenomenon is called Met-dependence. It is one of research focuses in the past decades that Met starvation is made artificially in tumor-bearing body to inhibit the tumor growth based on the therapy of Met-dependence. There are several methods to produce Met starvation: (1) degradation of Met by methioninase; (2) fast plus Met-depleting total parenteral nutrition (Met--TPN). Due to its unsatisfactory specificity methioninase can degrade Met irreversibly but it can also degrade Hcy, homocysteine, cysteine and cystine during a short period of time so that the normal cells are hard to survive. Only through fast and Met--TPN may Met starvation be produced and have little impact on normal tissue cells.

**Met-dependence of tumor cells**

Studies in vivo and in vitro can be used to judge whether tumor cells have the property of Met-dependence and to explore the impact of Met starvation on the proliferation of tumor cells. The research in vivo is not easy to conduct because of many inevitable factors. The prominent characteristic of the research in vitro is that the cells cultured are not influenced by complex internal environment and the rule of cell life activity can be studied. The change of cell physiological functions under the influence of single or multi-factors may be further observed when culture conditions such as physical, chemical and biological factors are altered artificially. Goseki et al explored the relationship of tumor growth and Met-dependence by animal experiment. We have also done such work in the past years. The studies suggested that Met starvation in tumor-bearing animals could inhibit the proliferation of tumor cells. In the experiment in vitro of gastric cell line SGC-7901, we found that the percentages of SGC-7901 cells in S and G2M phases in the Met-Hcy⁻ medium were both obviously elevated and the percentage of G0G1 cells significantly declined in comparison with those in the Met-Hcy medium. The results meant that the cell cycle of SGC-7901 was inhibited in the G2M phase. The present study showed again that in vitro the proliferation of human primary gastric cancer cells in the environment of Met-Hcy⁻ had the similar results to that of human gastric cancer cell line SGC-7901. The cell cycle of tumor cells was disturbed and the amount of cancer cells decreased significantly; however, no same phenomenon was observed in the gastric mucosa epithelial cells. The results suggested that human gastric cancer cells were Met-dependent. The cause of Met-dependence of tumor cells remains unclear. As far as we know now, the causes include: (1) decreased amount and activity of Met synthetase; (2) deficiency of 5-methyltetrahydrofolate reductase; (3) inability to make use of endogenous Met; (4) rise in the rate of basal transmethylation; (5) activated EJ/T24HRAS1 oncogene leading to the expression of Met-dependence.

**Significance of Met starvation in combination with chemotherapeutic drugs**

Chemotherapy can not be replaced by surgery and radiotherapy in the treatment of malignancies. At present most chemotherapeutic drugs are phase-specific, despite some without phase-specificity, all of which are hard to kill tumor cells completely. So the aim of tumor therapy is to take measures to improve the effect of chemotherapy. It is a new trial to drive the G0G1 cells to enter into the proliferating phase. Our previous clinical study and tumor-bearing rat experiment showed that after the treatment of parenteral nutrition, the percentages of S and S+G2M gastric cancer cells increased obviously while that of G0G1 cells decreased dramatically. Met deprivation can produce Met starvation in Met-dependent tumor cells, then metabolism of cancer cells is suppressed and more cells remain in the phases of S and G2. As a result, the effect of phase-specific chemotherapeutic drugs is enhanced significantly. This provides a new way to improve the therapeutic effect on tumors by using Met--TPN combined with chemotherapeutic drugs. Many authors have made the researches, both experimentally and clinically, on Met--TPN in
combination with chemotherapeutic drugs to improve the effect of chemotherapy. They confirmed that Met-TPN in combination with 5-FU, DDP and ADM respectively can enhance the sensitivity of chemotherapy of gastric cancer, breast cancer and Yashida sarcoma. Our present study supported the above results. It is hypothesized that Met starvation could make primary gastric cancer cells blocked in the phases of S/G2M. Theoretically, the more numerous are the cells in the proliferating phase, the stronger effect of phase-specific chemotherapeutic drugs have been produced.

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