

Interleukin-12 prevents colorectal cancer liver metastases in mice

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Abstract: Interleukin (IL)-12 has emerged to be a prospective molecule for antitumor therapies with many types of cancers. Here we examine the effect of IL-12 treatment in preventing the colorectal cancer liver metastasis in a mice model. At different doses, we found that IL-12 treatment decreased the formation of liver metastasis sites and the percentage of metastasis volume in the liver. Additionally, this treatment leads to improved survival function of mice after tumor cell transplantation. We believe that IL-12 based therapy provided a novel treatment to colorectal cancer patients suffering from liver metastasis.

Keywords: IL-12, liver metastasis, colorectal cancer, therapy

Introduction

Colorectal cancer has been one of the leading causes of mortality by cancer in recent years.¹⁻⁴ Liver metastases are common for colon cancer patients even after surgery, with poor prognosis.⁴⁻⁷ The treatments for existing liver metastasis or prevention of in vivo cancer metastasis are therefore important to improve the life quality and survival of these patients.

Interleukin (IL)-12 has emerged to be a prospective molecule for antitumor therapies.⁸⁻¹¹ IL-12 showed its effects in antagonizing or negatively modulating different types of cancers, as diagnosing factors;¹⁰⁻¹⁴ while its potential in treating the colorectal cancer liver metastasis has not been fully explored.^{8,15-17} In the present study we investigated the possibility of IL-12 based treatment to the colorectal cancer liver metastasis in a mice model.

Materials and methods

Animals

130 Balb/c mice (male, 9–10 weeks) were obtained from and housed in the animal center of Guangzhou Medical College (Guangzhou, People's Republic of China). This study was approved by the Ethics Committee of Animal Research in Guangzhou Medical College.

Colorectal cancer liver metastasis model

CT-26-murine colorectal adenocarcinoma cells (Shengao Shengwu, Shanghai, People's Republic of China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) (Shenggong Biotech, Shanghai, People's Republic of China) as previously described in Xu et al,¹⁵ at 37°C in 95% O₂ and 5% CO₂.

The animals were injected with 0.2 mL of 1×10^6 per mL CT26 cells via peritoneal cavity spleen transplant. Every 4 days (starting on the day of transplantation), the mice

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received 0.9% saline (control group, 20 mice), or 0.2 μg (40 mice), 0.5 μg (35 mice), 1 μg (35 mice) of recombinant murine IL-12 (Sigma and Shenggong Biotech, Shanghai, People's Republic of China) injection.

Measurements

10 days after treatment, 3 mL blood samples were obtained from the tail vein. The serum was immediately isolated with the centrifuge and the circulating interferon-gamma (IFN- γ) levels were detected by ELISA kits (Shunhe Biotech, Shanghai, People's Republic of China).¹⁵

20 days after the injection of CT26 cells, the animals (10 from each group) were sacrificed and the number of hepatic metastases were counted. The ratio of metastasis volume over the whole liver was calculated.

For other groups of animals, the treatment lasted until the death of the animals (maximum 90 days) for survival rate calculation.

Statistics

The data were represented as mean \pm standard deviation (SD), and analyzed with SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). The Student's *t*-test was used to compare intergroup differences and $P < 0.05$ was determined as statistically significant.

Results

IL-12 treatment lead to decreased metastasis

We found that IL-12 treatment decreased the number of liver metastasis sites in the liver as shown in Figure 1. In the control group there were 19 ± 4 sites, while in the 0.2 μg , 0.5 μg or

1 μg IL-12 treatment groups there were 9 ± 2 , 8 ± 2 , and 7 ± 3 sites respectively ($P < 0.01$ to control) (Figure 1).

IL-12 treatment also decreased the percentage of the overall liver with metastasis volume (Figure 2). In the control group the percentage was $77.2\% \pm 9.2\%$, while in the 0.2 μg , 0.5 μg or 1 μg IL-12 treatment groups were $70.4\% \pm 4.3\%$, $61.9\% \pm 4.8\%$, and $62.3\% \pm 6.4\%$, respectively ($P < 0.05$, 0.01 and 0.01 to control).

IL-12 treatment increased the survival rate of animals

We found that IL-12 treatment significantly increased the survival time length of the animals (Figure 3). Black: control, Red: 0.2 μg , Blue: 0.5 μg , and Cyan: 1 μg (Figure 3).

IL-12 treatment increased the blood circulating level of IFN- γ

We found that IL-12 treatment significantly increased the circulating IFN- γ level. In control group: $16.2 \pm 4.1 \mu\text{M/L}$; 0.2 μg group: $36.1 \pm 6.7 \mu\text{M/L}$ ($P < 0.01$); 0.5 μg group: $38.0 \pm 2.9 \mu\text{M/L}$ ($P < 0.01$); 1 μg group: $30.3 \pm 5.6 \mu\text{M/L}$ ($P < 0.01$).

Discussion

Many colorectal cancer patients demonstrated liver metastasis after the surgery, and contributed to the major cause of death in patients at advanced stages. The management of liver metastasis often includes surgical removal, chemotherapy and other biological treatments. Different types of molecules, such as immunomodulating cytokines, have been adopted to treat liver metastasis.^{18–21} In the present study we demonstrated that IL-12 treatment could effectively decrease the formation of

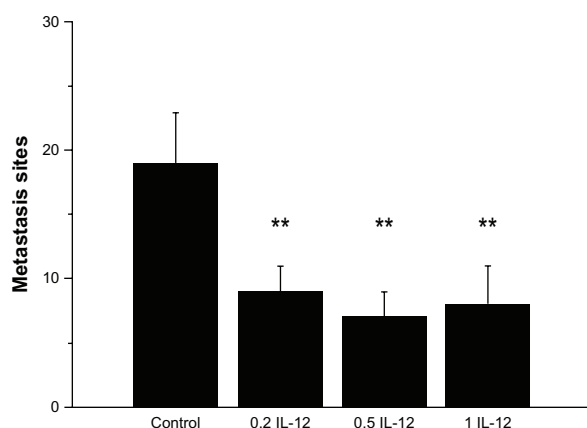


Figure 1 0.2 μg , 0.5 μg or 1 μg IL-12 treatment decreased the number of metastasis sites in the liver.

Note: **for $P < 0.01$.

Abbreviation: IL, interleukin.

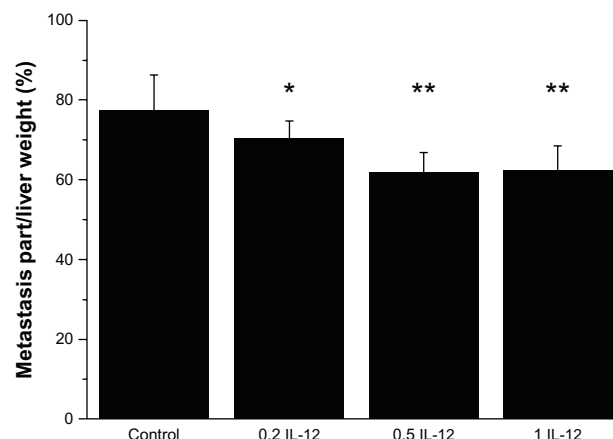


Figure 2 0.2 μg , 0.5 μg or 1 μg IL-12 treatment decreased the percentage of metastasis volume in overall liver.

Notes: *for $P < 0.05$; **for $P < 0.01$.

Abbreviation: IL, interleukin.

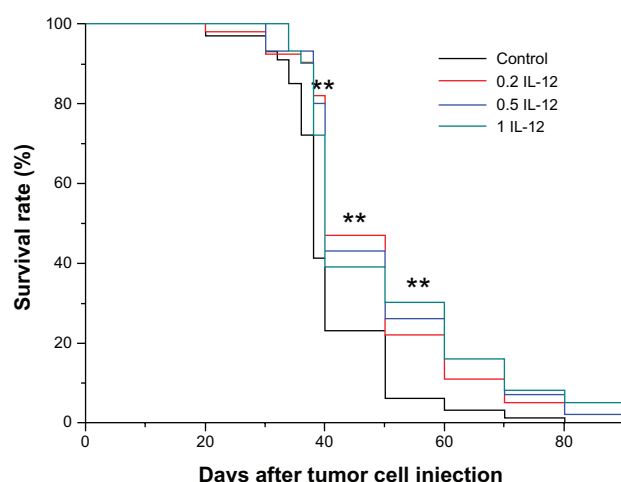


Figure 3 0.2 μ g, 0.5 μ g or 1 μ g IL-12 treatment increased the survival rate of animals.

Note: **shows all three groups were different from the control ($P < 0.01$).

Abbreviation: IL, interleukin.

liver metastasis sites and the percentage of metastasis volume in the liver. Additionally, this treatment leads to improved survival function of mice after tumor cell transplantation.

IL-12 is involved in the differentiation of naïve T cells into Th1 cells, and stimulates the growth and function of T cells. IL-12 also stimulates the production of tumor necrosis factor- α (TNF- α) and IFN- γ ,^{8,22,23} which is consistent with our present findings. This might mediate the beneficial effects of IL-12 treatment in preventing liver metastasis as IFN- γ could be anti-angiogenic and block new blood vessel formation through inducible protein-10.^{22,24,25} These are potential molecules with combined pharmacological effects when targeted together with IL-12.

It should be noted that IL-12 administration could also lead to some side effects and adverse results. Several solutions are possible: (1) combined treatments could be taken to prevent these adverse reactions; (2) controlled release of IL-12 in a slow manner might be of some help;¹⁶ (3) targeting the upstream/downstream signaling of IL-12 might lead to more physiological regulation of IFN- γ , yet the therapeutic effects are to be tested.

In conclusion, here we reported that IL-12 administration could be effective for colorectal cancer liver metastasis in a mice model. We expect to see the use of controlled release IL-12 for clinical treatment of colorectal cancer liver metastasis patients in the future.

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analyzed the data and wrote the paper; all authors approved the final manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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