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THERAPEUTIC EFFECTS OF rhTNF ALONE AND IN COMBINATION WITH KENGSHENGMYCIN IN EXPERIMENTAL HUMAN OVARIAN CANCER

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Based on experiments *in vitro*, we examined the therapeutic effect of rhTNF both alone and in combination with Kengshengmycin (KSM) on human ovarian cancer transplanted in nude mice. The results showed a considerable antitumor effect of rhTNF when it was used alone and a marked synergistic effect when it was used in combination with KSM on the xenograft tumors.

Key words: TNF, Ovarian tumor, Nude mice, Chemotherapy.

Ovarian cancer is always discovered too late in its course because of its propensity for occult and spread throughout peritoneal cavity with ease. In these advanced cases of ovarian cancer, even after extensive surgical debulking, tumor residuals remain in the abdomen to be treated with following chemotherapy.[1] Unfortunately, the current chemotherapeutic regimens get only a limited therapeutic effect by reason of the toxic side effects on patients that chemotherapeutic agents inherently have. Therefore, there is a need for novel and more effective therapeutic approaches. Recently, efforts have been focused on immunotherapy. Tumor necrosis factor (TNF) is a promising immunotherapeutic agent. The development of recombinant DNA technology has made it possible to produce a sufficient amount of recombinant human TNF (rhTNF) for experimental and clinical investigations. It has been reported[2,3] that rhTNF shows various degrees of antitumor effects on some human cancers. Our previous study[4] on human ovarian cancer cells *in vitro* also showed that there was a apparent cytotoxicity when rhTNF was used alone and a synergistic effect when it was used in combination with the chemotherapeutic agent Kengshengmycin (KSM). In the present study, we examined the therapeutic effects of rhTNF both alone and in combination with KSM on human ovarian cancer transplanted in nude mice.

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MATERIALS AND METHODS

Reagents

rhTNF with a specific activity of 6×10^6 U/mg was provided by the Beijing Institute of Basic Medical Sciences. It was diluted in phosphate-buffered saline just before used. KSM was purchased from Xinya Farmaceutical Factory (Shanghai, China) and it was dissolved and diluted with saline.

Tumor Cell Line

Human ovarian cancer cell line $OVCAR_3$ was provided by the Gynecology Research Laboratory of the Peking Union Medical College Hospital. The cells were incubated in RPMI-1640 medium (GIBCO, USA) supplemented with 10% newborn calf serum at 37 ℃ in a humidified atmosphere composed of 95% air and 5% CO_2.

Animals

Balb/c (nu/nu) female nude mice aged 6–8 weeks were obtained from the Cancer Institute of the Chinese Academy of Medical Sciences. They were bred and maintained in circumstances free of specific pathogen.

Xenografts and Treatments

All nude mice received ^{60}Co whole body irradiation of 2 Gy for three times within 10 days before transplantation. A human ovarian tumor originally transplanted into a nude mouse by subcutaneous injection of 7.6×10^6 $OVCAR_3$ cancer cells suspended in 0.2 ml of Hanks' balanced salt solution was used to establish 24 nude mouse models of xenograft tumors. Small cubes (3 mm^3) of fragment of the tumor were transplanted subcutaneously into the dorsam of the nude mice by surgery. Seven days after transplantation, when the tumor growth was still invisible, the mice were randomly divided into four groups of 6 mice each and daily treated as follows: Group A (the control group): i.p. injection of 1 ml of normal saline; Group B: i.p. injection of 3×10^4 U rhTNF and i.v. injection of 0.2 μg KSM; Group C: i.p. injection of 3×10^4 U rhTNF; Group D: i.v. injection of 0.2 μg KSM. The treatments were performed for 4 days per week and continued for 8 weeks. The tumor sizes were measured weekly by two dimension, and the tumor volumes were calculated with the formula[5]: $V=L\times W^2/2$, where L is the length of the tumor and W is the width.

Morphologic Observation

Four days after the final therapy, all the nude mice were sacrificed by cervical dislocation and autopsies were performed. For histological examination, tumor tissue specimems from xenografts in four groups were fixed in 10% buffered formalin, embeded in paraffin, and processed by standard methods. Sections were stained with hematoxylin-eosin and observed under light microscope.

Data Analysis

Statistical determination was made with Wilcoxon rank sum analysis.

RESULTS

Therapeutic Effects of rhTNF Alone and in Combination with KSM on Xenografts in Nude Mice

After 5 weeks of treatment, the mean tumor volume in the nude mice treated with rhTNF was found to be considerably smaller ($P<0.01$) than that in the control group, as shown in the Figure 1. A marked synergistic therapeutic effect was found in the group treated with rhTNF in combination with KSM, whereas treatment with KSM alone showed no therapeutic effect in this experiment.

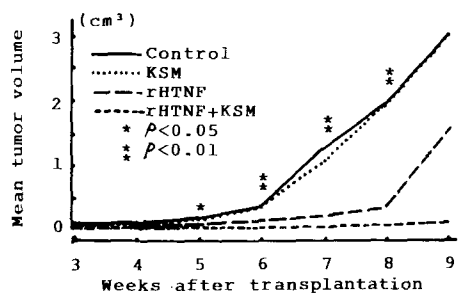

Fig. 1. Growth curves of human ovarian cancer $OVCAR_3$ transplanted in nude mice in relationship to different treatment.

Morphologic Changes in Relationship to Treatments

In all of the four groups mice, the xenograft tumors were located in the dorsam subcutis of the mice where originally transplanted and wrapped in the false membranes, without evidence of distant metastasis. No abdominal distension was found in all the mice tested. Light microscopy showed that the

histologic pattern of the xenograft tumors from the control group mice was similar to that seen in the fresh specimen of poorly differentiate adenocarcinoma of human ovary. In contrast, the tumor in the mice treated with rhTNF and rhTNF/KSM necrotized, with evidence of hemorrhage, infiltration of neutrophils and fibrosing. These histologic changes were especially serious in the group treated with rhTNF in combination with KSM, while that of the group treated with KSM alone were very slight.

DISCUSSION

Tumor necrosis factor, first identified in 1975 by Carswell and associates in tumor-necrotizing mouse serum, has attracted much attention because of its specific antitumor activity while sparing normal tissue cells. Since 1985 when large amounts of highly purified rhTNF became available owing to the recombinant DNA techniqe,[6] many types of human cancers, including lymphoma, sarcoma, and renal and colorectal cancers have been reported to be sensitive to rhTNF. The data presented here and previous[4] show that the antitumor potential of rhTNF on human ovarian cancer is promising both *in vitro* and *in vivo*. In an attempt to improve the efficacy of treatment and to avoid the serious side effects of high doses rhTNF in clinical trials, the use of low doses rhTNF in combination with various chemotherapeutic or immunotherapeutic agents has been considered. We have recently showed[4,7] that the combination of rhTNF with KSM, cisplatin and recombinant human interferon has some synergistic antitumor activities on human ovarian cancer cells *in vitro*. The present study further demonstrate that the combination of rhTNF with KSM has a synergistic therapeutic effect on human ovarian cancer transplanted in nude mice. KSM is a common chemotherapeutic agent widely used for the treatment of ovarian cancer in China. Its structure and function are similar to those of actinomycin D (Act. D) which is commonly used abroad as an antitumor chemotherapeutic agent. Alexander and associates[8] have studied thoroughly the synergistic cytotoxicity of rhTNF with Act. D on L_{929} cell line and concluded that the synergistic enhancement of Act. D by TNF was related to topoisomerase-mediated DNA damage, but not to other forms of DNA damage.

Since 1969 when Rygaard first reported the successful transplantation of human cancer to nude mouse, the xenograft model system has been widely used to assess the antitumor activities of various drugs, because it is relatively easy for human malignancies to grow in nude mice, while maintaining their original morphologic characteristics. The validity of the transplants of human cancers in nude mouse as a predictive system for testing new antitumor agents and determining optimal treatment schedules has been reported.[9] Based on experiments *in vitro*,[4] we examined the *in vivo* antitumor activities of rhTNF, both alone and in combination with KSM, on human ovarian cancer $OVCAR_3$ transplanted to nude mice. The results showed a considerable antitumor effect with rhTNF alone and a marked synergistic effect with rhTNF combined with KSM on the xenograft tumors. The antitumor mechanisms of TNF *in vivo* are complicated.[1,2] Besides its direct effects on tumor cells, TNF also acts on tumor through the immune response system of the host. Recently, Manda[10] reported that the antitumor effects of TNF are more due to the damage of newly formed fine capillaries in the tumor. This special mechanism of action of TNF may be one explanation of the phenomenon in the present study that the tumor growth in the rhTNF group was markedly inhibited in the early stage of treatment and the inhibition decreased after 7 weeks of treatment, as shown in the Figure. The therapeutic effects are also associated with the route, timing and sequence of the administration of drugs when TNF is used in combination with the chemotherapeutic agents.[10]

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