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# Anti-tumor Activity of Shark Cartilage Extract and Related Mechanisms*

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**Abstract** Shark cartilage powder and extract made in our laboratory, referred to SCE(shark cartilage extract), were used to investigate the effects of shark cartilage on the tumor cell lines HeLa and BEL-7404, tumor-bearing mouse $S_{180}$, Heps, EAC, and several types of immune cell. The results have shown that SCE significantly inhibits tumor growth, reducing tumors rate by 60%-70%. In addition, the activities of natural killer (NK) cells and interleukin-activated killer (LAK) cells to the target cells $Yac_{-1}$ and $P_{815}$ are increased after treatment with SCE, suggesting a possible mechanism for increasing tumor monitoring and inhibition activities.

**Key words** shark cartilage; anti-tumor; immune function

Shark is one of the rare species on the surface of earth that is free of cancers either in its natural environment or when exposed to a large amount of carcinogens. It is a prominent representative of cartilaginous fish which is very scarce compared to bony fish. Shark skeleton is composed entirely of cartilage, which is able to function without connecting blood vessels, lympho-system and nerve system, and is rarely attacked by cancerous cells. Many types of shark cartilage products have been made and their anti-tumor efficacy is significant in some clinical studies[1]. The shark cartilage extract (SCE) made in our laboratory, was used to conduct a large scale research on mouse tumor models, cultured tumor cell lines, immune cells, and organs to understand its anti-tumor activity and mechanisms.

## 1 Materials and Methods

### 1.1 Materials and reagents

SCE was made in our laboratory. Shark cartilage powder (USELT) was made by USA. Tumor-bearing mouse $S_{180}$, Heps, EAC and tumor cell lines HeLa, and BEL-7404 were obtained from the Cancer Institute, Chinese Medical Academy of Sciences. Mouse $C_{57}$BL/6, and BALB/C were obtained from the Institute of Zoology, Chinese Academy of Sciences. CTX was purchased from the 12th Pharmaceutical Factory in Shanghai. 5-Fu was purchased from the Nantong Pharmaceutical Factory. γIL-2 was obtained from the Institute of Biology and Engineering, Military Medical Academy of Sciences.

### 1.2 Methods

#### 1.2.1 Production of SCE

SCE was made in our laboratory according to a modified version of the method of Irwin W. Lane, Short Hills, NJ.

#### 1.2.2 Effects of SCE on mouse tumor model $S_{180}$, Heps, and EAC

**Implanting** The mouse tumor tissues were separated and their cells were kept at a density of $5 \times 10^5$ cells/mL in sterilized 0.9% NaCl. 0.2 mL of this solution was implanted into the axilla (solid tumor), and the abdominal cavity (ascitic) of each mouse.

**Grouping** Tumor-bearing mice were grouped

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at random. Four experimental groups received doses of 100, 200, 400 mg/kg B.W. of SCE. Controls were given by the corresponding solvent, while positive controls received CTX, 5-Fu, and USELT.

**Evaluation of efficacy** (1) For solid tumors, the tested reagents were given to the animals for 8 d. The animals were sacrificed 24 h after the last injection. The tumors were separated from the surrounding tissues and weighted. The rate of tumor reduction was calculated as:

rate of tumor reduction = [(tumor mass in control group − tumor mass in experimental group)/tumor mass in control group] × 100.

(2) For EAC, the tested reagents were given to the animals for 8 weeks. The average survival rate of the animals in both the control and experimental groups was recorded. The survival rate was calculated as:

survival rate = [(average survival days in control group − average survival days in experimental group)/average survival days in control group] × 100.

### 1.2.3 Inhibition of growth of cultured tumor cell lines

HeLa and BEL-7404 cells were incubated with different concentrations of SCE for 72 h. $^{3}$H-TdR was then added to the cultures and the incorporation (counts per minute, *c*/min) of the $^{3}$H-TdR was measured by a liquid scintillation counter. The inhibition of the DNA synthesis rate was calculated as:

inhibition rate of DNA synthesis = [(*c* in control group − *c* in experimental group)/*c* in control group] × 100.

### 1.2.4 Effects on the activities of LAK cells and NK cells

(1) Stimulation of LAK and NK cells

6-8 week-old $C_{57}$BL/6 mice and BALB/C mice were used to measure the activities of LAK cell and NK cell, respectively. The animals were sacrificed and the spleens were then separated. Single spleen cells were separated and suspended in the 1640 medium containing 10% calf serum at a density of $2 \times 10^{6}$ cells/mL. rIL-2 (500 IU/mL) was used to stimulate the LAK cell activity. The activity was measured after the cells were incubated at 37 ℃ with 5% $CO_2$ for 5 d.

(2) Activity measurement

The LDH-4h dilution procedure was used to measure the activities of both LAK cells and NK cells. The cell ratio of LAK cell to the target cell $P_{815}$ used to measure the LAK cell activity was 50∶1. The cell ratio of NK cell to the target cell Yac-1 used to measure the NK activity was 200∶1. Each sample had three duplicated wells. Both the maximum releasing wells and the natural releasing wells served as controls. The cells were incubated at 37 ℃ with 5% $CO_2$ for 4 h. The *c*/min produced from $^{51}$Cr was measured using a $\gamma$-counter. The killing percentage was calculated as follows:

killing percentage = [(*c* from experimental well − *c* from natural releasing well)/(*c* from maximal releasing well − *c* from natural releasing well)] × 100.

Four experiment groups received stimulation as described above. The SCE concentrations 0.5, 1, 2, 4 g/L used in the four groups were used to detect the effect on the killing activities of LAK cells and NK cells.

## 2 Results

### 2.1 Anti-tumor activity of SCE

#### 2.1.1 Effects of SCE on mouse solid tumor model $S_{180}$ and Heps

Tumor bearing mice received different dose of SCE and USELT for 8 d. The animals were sacrificed 24 h after the last injection. The tumors then were weighted. Figure 1 shows all doses of SCE can inhibit the growth of the solid tumor, $S_{180}$ and Heps. The inhibitory rate can reach 74.65%. A dose-response relation can be seen in Fig. 1. The experiment shows USELT has less inhibitory effect compared to SCE at the same dose (not shown in Fig. 1).

#### 2.1.2 Effect of SCE on the mouse ascitic tumor model EAC

The tested reagents SCE, USELT were given to tumor bearing mice for 8 weeks. The average survival days of the mice in both the control and experimental group were recorded as shown in Fig. 2. The experiment shows SCE prolongs the average survival time in the EAC mouse tumor model, the maximum survival rate is 64.75% vs. control. USELT still has less effect than SCE.

### 2.2 Effects of SCE on the activities of LAK and NK cells

LAK, NK cells obtained from BALB/C mouse were mixed with various concentrations of SCE. The activity of NK cell and LAK cell determined

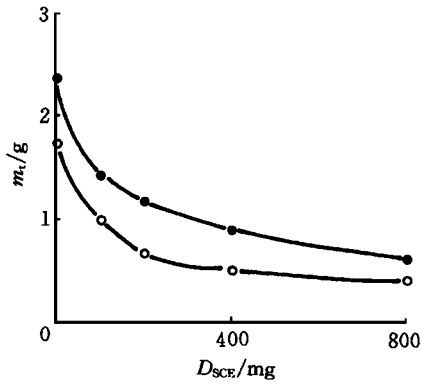


**Fig. 1 Inhibitory effects of SCE on mouse solid tumor model $S_{180}$ and Heps**
●, mouse solid tumor model-$S_{180}$;
○, mouse solid tumor model-Heps

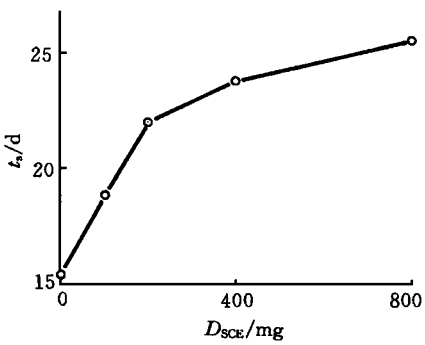


**Fig. 2 Effect of SCE on survival time of the mouse ascitic tumor model**

by the LDH-4h dilution procedure after the 5 d incubation is shown in Fig. 3. Clearly, SCE significantly increased not only the killing activity

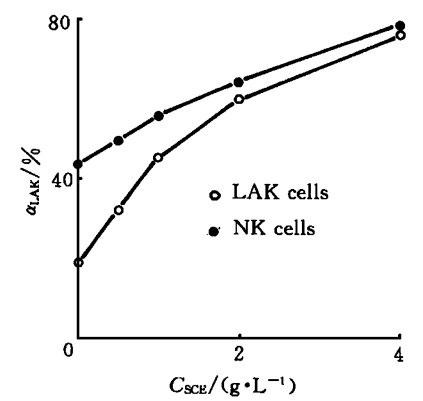


**Fig. 3 Effects of SCE on the killing activity of NK cells and LAK cells**

of LAK cell to target cell $P_{815}$, but also the killing activity of NK cell to target cell Yac-1. In the rIL-2/LAK system, SCE was able to induce higher LAK cell activity than that induced by rIL-2 only.

### 2.3 Inhibition of cultured tumor cell lines by SCE

At a concentration of 2 g/L in the culture medium, SCE significantly inhibited the DNA synthesis of HeLa(61.35%, $P < 0.05$) and BEL-7404 (53.28%, $P < 0.01$) cells, compared to the DNA synthesis in the controls.

## 3 Discussion

The anti-tumor efficacy of shark cartilage has been proved in many investigations. Efforts have been made to isolate and purify the active components of shark cartilage, but the procedure still faces some obstacles, and its anti-tumor mechanism remains unclear. In 1990, an active component with a relative molecular mass of $10^3$-$10^4$[2] that inhibited the growth of blood vessels was purified using the guanidine extraction and acetone precipitation procedure developed by Oikawa et al. Using similar procedures, Chinese researchers isolated another positively charged glucosylated protein with a relative molecular mass of 16 100 and pI 8.15[3]. This protein is able to restrict the growth and migration of blood vessels, thereby, interrupting the nutrient supply to the tumor as well as the metastasis pathway. It also has been indicated that products of shark cartilage can directly inhibit the growth of some tumor cell lines *in vitro*[4]. Other evidence has shown that shark cartilage may play a role in reducing mutation and generation of reactive oxygen species[5], as well as inhibiting the production of 6-Keto-PGF$_{1\alpha}$ in tumor-carrying mouse[6] and stimulating the immune system[4]. More than seven anti-tumor factors have been isolated from shark cartilage[7] so far. In the present study, we observed distinct anti-tumor activity of SCE made in this laboratory. The inhibition of tumor growth by SCE indicated a dose-response relation with an inhibition rate as high as 74.65% in mouse tumor model $S_{180}$, 73.79% in Heps, and 64.75% in EAC. It is interesting that the effect of SCE on the growth of EAC was less than on $S_{180}$ and Heps, which suggests a possible selectivity of the tumor type related to their different dependence on the blood vessels. Our study has also shown that SCE

is able to inhibit the growth of tumor cell lines HeLa and BEL-7404 by reducing their DNA synthesis, with inhibition rates from 50% to 60%. This may reflect the existence of tumor inhibition factors in SCE which can directly kill cultured tumor cells.

NK cells belong to a family of heterogeneous multifunction cells. As the first line of defense for the tumour monitoring function of the immune system, NK cells have a broad spectrum of activity for killing tumor cells belonging to the same lineage and same or different species. The activity of NK cells does not depend upon the function of antibodies, since Interleukin-2, Interferon-$\gamma$, and Interferon-$\alpha$ are able to enhance its activity. In the present study, SCE significantly increased the activity of NK cells from the spleen of BALB/C mice, suggesting the possible involvement of SCE in the anti-tumor function. In addition to being similar to NK cells, LAK cells are capable of killing NK resistant solid tumor cells. The activity of LAK can only be induced by some cytokines, such as rIL-2. The rIL-2 and LAK combination currently serves as an effective chemotherapy for cancer treatment. SCE significantly increased the activity of LAK cells to the target cells $P_{815}$, not only clarifying the anti-tumor mechanism, but also laying an experimental foundation for designing a better combination therapy in clinical applications.

In the present study, SCE, a crude extract of shark cartilage, has shown obvious anti-tumor activity. SCE exhibited higher activity than common chemotherapeutic medicines, such as CTX and 5-Fu. These results suggest the potential application of SCE in cancer treatment. We have isolated two small anti-tumor proteins from the shark cartilage and obtained their partial N-terminal amino acid sequences. Further molecular biology studies are currently being conducted to understand the anti-tumor mechanisms of these two proteins.

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