Study on Effect of Kanglaite Injection (KLT) on the Expression of Fas/Apo-1, FasL and PCNA in Renal Carcinoma Cell Lines
Wang Junjie, Sun Xinchun, Sheng Wenjiang, Yu Lizhang

[Abstract]
Objective: Investigate the effect of KLT on the expression of Fas/Apo-1, FasL and PCNA genes in renal carcinoma cell line (GRC-1).

Materials and Method: The expression of Fas/Apo-1, FasL and PCNA genes in renal carcinoma cells was examined by immunocytochemical method.

Results: The Label Index (LI) of Fas/Apo-1 gene in GRC-1 cells was 74.6% and that in blank emulsion group was 21.2%. GRC-1 cells in 0.2mg/ml KLT group and in blank emulsion group did not have change in FasL expression. LI of PCNA in GRC-1 cells was 15.2% in KLT group and almost no expression in blank emulsion group.

Conclusion: KLT can increase Fas/Apo-1 and PCNA gene expression of renal carcinoma cell lines and FasL gene expression did not show any change.

[Key Words] KLT, Fas/Apo-1, FasL, PCNA

Fas/Apo-1 is a kind of crucial signaling molecules. Studies in recent years showed that it plays an important role in inducing apoptosis. The effect of cytotoxic T-cell (CTCS) and natural killing cell (NK) on killing tumor cells mainly relies on membrane protein FasL that binds tumor cell with Fas/Apo-1 and induces cell apoptosis. Therefore, some scholars have attempted to make new trial in tumor biology and immunotherapy through this channel [2]. KLT is an antitumor preparation extracted from traditional Chinese medicine coix seed, which is proved by clinical application study that it can remarkably inhibit some tumors and has exact efficacy. However, its action mechanism is not explicit yet. The paper discussed the molecular biology mechanism of anticancer KLT by means of renal carcinoma cell strain to provide theoretical guidance for clinical tumor comprehensive treatment.

Materials and Method
1. Drug and cell culture
KLT (10%, batch No. Z-108) and blank emulsion are provided by Zhejiang Kanglaite Pharmaceutical Co., Ltd.

Biotin labeled GAR IgG and LSAB compound are provided by American Vector Biological Reagent Company. Fas/Apo-1 and FasL are polyclonal antibody of rabbit anti-human primary antibody with immunoaffinity purification. PCNA is monoclonal antibody of mouse anti-rat (product of American Santa Cruz, provided by Beijing Zhongshan Biotechnology Co., Ltd.). DAB is product of Swiss Fluka Biochemical Reagent Company.
Renal granular cell carcinoma strain (GRC-1) is provided by Urology Surgery Research Institute in our hospital. The culture medium RPMI-1640 contains 15% calf serum and the strain is cultured in 37°C, 5% CO₂ incubator with conventional passage cultivation.

2. Chemical analysis of immunocyte
The exponential phase cells are treated with 10μl/ml blank emulsion and 0.2mg/ml KLT for 48h and digested by 0.25% pancreatin and 0.02% EDTA. Cells are re-suspended with culture solution, counted and adjusted to appropriate concentration for smear. Smear is placed in wet box at 37°C for 5hr., washed with PBS twice, fixed by cold acetone for 15min, and then put into fridge at 4°C for reservation.

Dyeing of immunocyte adopts SP™. All specimens adopt LASB method under the same conditions for immunocyte dyeing. Primary dilution is 1:50. Meanwhile, substitute the primary separately with PBS and normal rabbit serum for blank control.

Method to identify gene expression strength: Positive gene expression of Fas/Apo-1 and FasL is claybank dyeing of cell membrane; and that of PCNA is expressed as claybank granule dyeing of cell nucleus. The positive developing ones can be divided into three grades namely strong, medium and weak as per dyeing strength of cell membrane. Select 5 high-power fields (×400) as viewing area. Calculate the mean of cell quantity in 5 fields. The labeling rate (LI%) of each gene in cytoplasm and cell nucleus is calculated in accordance with the following formula:

\[
LI\% = \frac{\text{Number of positive cells}}{\text{Total number of marked cells}} \times 100\%
\]

Results
1. Effect of KLT on gene expression of Fas/Apo-1 and FasL in GRC-1 cells
From Tab. 1, Fig. 1 and Fig. 2, it can be seen that in blank emulsion group the gene expression strength of Fas/Apo-1 in renal carcinomas is comparatively weak with LI as 21.20% while the gene expression strength of Fas/Apo-1 in KLT group is enhanced with LI as 74.60%. There is nearly no FasL gene expression in cytoplasm of kidney carcinoma in blank emulsion group. There is no significant change in FasL gene expression of GRC-1 cells in KLT group.
Fig. 1 Comparison in Fas/Apo-1 gene expression between blank emulsion group and Kanglaite group (×400). Fas/Apo-1 expression of GRC-1 cytoplasm in blank emulsion group is less and strength is weaker; while Fas/Apo-1 expression of GRC-1 cytoplasm in Kanglaite group is stronger. Negative cell (→). Positive cell (⇒).

Fig. 2 Comparison in FasL gene expression between blank emulsion group and Kanglaite group (×400). There is almost no FasL gene expression of GRC-1 cytoplasm in blank emulsion group. There is no change in FasL gene expression of GRC-1 cytoplasm in in Kanglaite group. Negative cell (→).

2. Effect of KLT on expression of renal carcinomas PCNA
There is almost no PCNA gene expression of GRC-1 cell nucleus in blank emulsion group; PCNA gene expression of GRC-1 cell nucleus in Kanglaite group increases. LI is 15.20%. See
results in Tab. 1 and Fig. 3.

**Tab. 1 Effect of 0.2mg/ml KLT on Fas/Apo-1, FasL and PCNA gene expression of GRC-1 cells**

<table>
<thead>
<tr>
<th></th>
<th>Blank emulsion group</th>
<th>KLT group</th>
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<tbody>
<tr>
<td>Fas/Apo-1</td>
<td>21.2</td>
<td>74.6</td>
</tr>
<tr>
<td>FasL</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>PCNA</td>
<td>2</td>
<td>15.2</td>
</tr>
</tbody>
</table>

**Fig. 3** Comparison in PCNA gene expression between blank emulsion group and Kanglaite group (×400). There is almost no PCNA expression of GRC-1 cell nucleus in blank emulsion group; the PCNA gene expression of GRC-1 cell nucleus in Kanglaite group increases. Negative cell (→). Positive cell (⇒).

**Discussion**

Renal carcinoma is a kind of tumor that bears unsatisfactory treatment effect clinically. Due to MDR gene expression in about 87% of renal carcinoma, chemotherapy effect is not satisfactory. Currently there is no ideal therapy to improve efficacy of renal carcinoma.

Apoptosis is a suicide process that independently completed by cells, which has unique form and biochemical characteristics. Apoptosis plays a critical role in maintaining normal structure and functions of organs. Wang Junjie et al. [3, 4] have discovered that KLT can inhibit proliferation of renal carcinoma cells and induce their apoptosis. They also have proved that the apoptosis reached maximum value at dose of 0.2mg/ml. KLT has effect to modulate P53 and inhibit Bcl-2 gene expression in immunocytochemical findings. A variety of studies have
reported that KLT has significant killing effect on several tumor cells in vitro and in vivo \cite{5, 6}. Meanwhile, KLT is found to present immunoenhancing effect. Nevertheless its molecular-biological mechanism is not explicit yet.

Fas/Apo-1 and FasL play a key role in modulating apoptosis in immunosystem. Fas/Apo-1 is a kind of transmembrane protein, which is also known as Fas/Apo-1 protein, Fas/Apo-1 antigen or Fas/Apo-1 receptor. It is a member of NGF and TNF receptor super family. Fas/Apo-1 molecule is signal receptor of apoptosis, which activates apoptosis gene product and induces apoptosis of the cell that Fas/Apo-1 molecule exists after binding Fas/Apo-1 antibody or ligand (Fas Ligand, FasL) after special protein mediation in cytoplasm. Although the apoptosis signal of Fas/Apo-1 is not explicit yet, in partial cell lines, tumor cells can be killed by activating Fas/Apo-1. To destroy Fas/Apo-1 signal system can selectively escape immune monitoring to obtain survival advantage. On the surface of activated T-lymphocyte the two proteins have high expression while they are weak on other T-Lymphocytes. Fas/Apo-1 mediation apoptosis requires certain strength of Fas/Apo-1 antigen expression on cell membrane and complete link structure of apoptosis signal conduction system \cite{2}.

Fas/Apo-1 molecules are widely expressed in many normal tissues and tumor tissue cells. Strength of Fas/Apo-1 molecule expression in tumor tissue has crucial guidance effect in tumor treatment and estimation of prognosis. Study of Li Hongjun et al \cite{7} showed that Fas/Apo-1 had stronger expression in bladder cancer cell line (T24) and prostatic cancer cell line (PC-3M) while it became weaker or had no expression in bladder cancer cell line BIU-87 and renal carcinoma cell line RCC-949 and GRC-1. Our study also indicated that Fas/Apo-1 molecules have weak expression in GRC-1 cells so that they are able to escape FasL immune T-lymphocyte monitoring. It may be one of the reasons for the unsatisfactory efficacy in treating renal carcinoma with immunotherapy such as IL-2 and interferon-\(\gamma\). 48hr after treating with 0.2mg/ml KLT we discovered that it could up-regulate Fas/Apo-1 molecule expression in GRC-1 cell. Thus, it could be inferred that enhancement of Fas/Apo-1 molecule expression in GRC-1 cell help activated T-lymphocyte identify tumor cells more easily. The binding of FasL with Fas/Apo-1 could conduct apoptosis signal and induce tumor cell apoptosis.

PCNA is the subunit of DNA polymerase\(\delta\), as a kind of cell cycle dependent protein; it reaches maximum value at S phase. Research showed that PCNA expression was related to low tissue grading. Low PCNA expression in rectal cancer is significantly related to difference in tumor prognosis \cite{8}. Recently, studies showed that PCNA had function of repairing damaged DNA. It is proved by our study that PCNA expression got remarkably enhanced 48 hours after treating with 0.2mg/ml KLT. It is analyzed that PCNA had participated in the repairing process induced by KLT in GRC-1 cell DNA damage. When the damage degree exceeded repairing ability, other genes such as P53 and Bcl-2 would order cell entering apoptosis. Hence, in our opinion, the antitumor efficacy of KLT is the result of interaction, mutual-influence and
mutual-restriction of polygene. Giving full play to the antitumor efficacy of KLT to higher degree needs further exploration in its interaction with other genes to improve therapeutic effect in comprehensive tumor treatment.

References


[7] Li Hongjun, Yu Lizhang, Guo Yinglu. Expression of Fas/Apo-1 in urinary tumor cells. (To be Published.)