Modulatory Effects of EPA and DHA on Proliferation and Apoptosis of Pancreatic Cancer Cells

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Summary: In order to investigate the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the proliferation, apoptosis of pancreatic cancer cell line SW1990 cells and the expression of cyclin E mRNA, the SW1990 cells were treated with different concentrations of EPA or DHA (20, 40, 60 µg/mL) for 0, 12, 24, 36 and 48 h respectively. By using MTT method, the inhibitory effects of EPA or DHA on the cell growth were assayed. Real-time PCR was used to detect the expression changes of cyclin E mRNA after the SW1990 cells were treated with 40 µg/mL EPA or DHA for different time. Flow cytometry was used to test the changes of apoptotic rate in the SW1990 cells treated with different concentrations of EPA or DHA for 24 h. The results showed that EPA and DHA could inhibit the growth of SW1990 cells in a time- and concentration-dependent manner (P<0.01). EPA or DHA could also significantly inhibit the expression of cyclin E mRNA in a time-dependent manner (P<0.05). EPA or DHA could induce the apoptosis of SW1990 cells in a concentration-dependent manner (P<0.01). It was concluded that ω-3 fatty acid could inhibit the proliferation of pancreatic cancer cell line SW1990 cells and promote their apoptosis. The down-regulation of the cyclin E expression by ω-3 fatty acid might be one of the mechanisms for its anti-tumor effect on pancreatic cancer.

Key words: ω-3 fatty acid; cyclin E; apoptosis; malignant tumor

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main components of ω-3 fatty acid and belong to the essential fatty acids which are not synthesized by self. Epidemiological investigations revealed that in the population with dietary high ω-3 fatty acid intake the incidence of colon carcinoma, prostate cancer and breast cancer etc was obviously reduced[1]. Tanaka et al reported that EPA and DHA could inhibit the proliferation of Ehrlich ascite tumor cells by suppressing the DNA synthesis of tumor cells[2], but the study of ω-3 fatty acid on pancreatic cancer and the mechanism was little at present. In this study, the human pancreatic cancer cell line SW1990 cells were treated with different concentrations of EPA or DHA in order to investigate its effects on the proliferation and apoptosis of cells so as to provide the experimental evidence for EPA and DHA clinically treating pancreatic cancer.

1 MATERIALS AND METHODS

1.1 Main Reagents

- EPA and DHA (Sigma, USA); MTT (Janssen Chimica, USA); RPMI-1640 medium, fetal calf serum (FCS) and TRIzol (Gibco, USA). Real time PCR kit
- TRIzol (Gibco, USA); RPMI-1640 medium, fetal calf serum
- Affymetrix Human Genome U133 Plus 2.0 array (Shanghai Jiusheng Medical Product Co., Ltd., China); Annexin V/PI kit (Shenzhen Jingmei Biological Product Co., Ltd., China). Human pancreatic cancer cell line SW1990 (American Type Culture Collection, ATCC, USA). The SW1990 cells were routinely passaged and cultured in low-glucose DMEM medium containing 10% FCS, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a saturated humidified incubator with 5% CO₂.

1.2 Assay of Inhibitory Rate of Cell Proliferation by MTT

The SW1990 cells in logarithmic phase were digested with 0.25% trypsin, adjusted to a density of 5x10⁵/mL, and put in a 96-well plate. In each well, 100 µL cell suspension was added and treated with different concentrations of EPA or DHA (20, 40, 60 µg/mL, serving as 3 groups). Negative control group was set up by adding nothing. Each group was triplicate. After the cells were cultured for 0, 12, 24, 36 and 48 h, the wells were removed from the incubator, following by addition of MTT (5 mg/mL) 10 µL in each well, continuously cultured for other 4 h. After the cells were centrifuged and the supernatant was discarded, DMSO 100 µL was added into each well and the cells were thoroughly dissolved in 490nm. A value in each well was determined by ELISA. Growth inhibitory rate of tumor cells=(1-Everage A values in experimental group/A values in control group) x100%.

1.3 Detection of the cyclin E mRNA Expression by Real-time PCR

SW1990 cells were treated with 40 µg/mL EPA or
DHA for 0, 12, 24, 36 and 48 h respectively. The cells were harvested and the total RNA was extracted with TRIzol according to the instruction of the kit. The RNA was dissolved and its purity and concentration were measured by UV spectrophotometry (A$_{260}$/A$_{280}$>1.8). The primers were designed by Premier 5.0 software according to the sequence of cyclin E cDNA in Genebank and synthesized by Shanghai Shenggong Biologic Co., Ltd., China.

For cyclin E, up-stream: 5'-AATAGAGAGGTCTGG-3', down-stream, 5'-AGATAGTCAACCTGCATG-3'; For β-actin, up-stream, 5'-CGCTGCGCTGTCGTCGACT-3', down-stream, 5'-GTCACGCACGATTCCCAGCT-3'. The reaction conditions were as follows: pre-degeneration at 94°C for 5 min, degeneration at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 2 min, 45 cycles; final extension at 72°C for 10 min. cDNA-free negative control group was set up at the same time of amplification. The Ct values (threshold cycle) of the tested samples were measured by real-time PCR. The relative levels of cyclin E mRNA expression were taken as 2$^{-\Delta\Delta Ct}$, in which, $\Delta\Delta Ct=\Delta Ct_{\text{Negative controls}}-\Delta Ct_{\text{tested samples}}$.

1.4 Detection of Apoptosis by Flow Cytometry

SW1990 cells were treated with EPA or DHA (0, 20, 40, 60 µg/mL) for 24 h, harvested, washed with PBS buffer and resuspended. By using AnnexinV/PI-conjugated flow cytometry, the apoptosis was detected according to the instruction of the kit. The cells were adjusted to a density of 2×10$^5$/mL, and 190 µL PBS buffer, added with 5 µL PI, and assayed by flow cytometer (Becton Dickinson, USA).

1.5 Statistical Analysis

The experimental data expressed as $\bar{x} \pm s$ and tested by analysis of variance. The intergroup comparison was done by LSD method. All statistical analyses were performed by SPSS11.5 statistical software. $P<0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Effects of EPA and DHA on the Proliferation of SW1990 Cells

MTT results revealed that after the SW1990 cells were treated with different concentrations of EPA or DHA (20, 40 and 60 µg/mL) for 0, 12, 24, 36 and 48 h respectively, the proliferation of SW1990 cells could be inhibited by EPA or DHA in a time- and concentration-dependent manner. There was significant difference in the inhibitory rate between experimental group and negative control group ($P<0.01$, table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inhibitory rate (%)</th>
<th>F values</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EPA (µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>3.12±0.23</td>
<td>5.37±0.38</td>
<td>6.75±0.49</td>
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<tr>
<td>40</td>
<td>10.34±0.75</td>
<td>22.71±1.29</td>
<td>31.12±1.66</td>
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<tr>
<td>60</td>
<td>14.93±0.72</td>
<td>29.05±1.60</td>
<td>38.43±2.1</td>
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<tr>
<td>DHA (µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5.38±0.62</td>
<td>9.53±0.55</td>
<td>11.99±0.62</td>
</tr>
<tr>
<td>40</td>
<td>12.82±0.70</td>
<td>24.36±1.17</td>
<td>32.63±1.72</td>
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<tr>
<td>60</td>
<td>16.83±0.86</td>
<td>35.19±1.82</td>
<td>44.93±2.31</td>
</tr>
</tbody>
</table>

$P<0.05$, intergroup comparison in the experimental groups

2.2 Effects of EPA and DHA on the Expression of cyclin E mRNA in SW1990 Cells

After the SW1990 cells were treated with EPA or DHA (40 µg/mL) for 0, 12, 24, 36 and 48 h respectively, the expression of cyclin E mRNA was down-regulated in a time-dependent manner. There was significant difference between experimental groups and negative control group ($P<0.05$, fig. 1).

2.3 Effects of EPA and DHA on the Apoptosis of SW1990 Cells

After the cells were treated with 20 µg/mL EPA or DHA for 24 h respectively, there was no significant difference in the apoptotic rate of SW1990 cells between the experimental group and negative group. But when the EPA or DHA concentration was increased to 40 and 60 µg/mL, EPA or DHA could induce the apoptosis of SW1990 cells in a concentration-dependent manner, and there was significant difference in comparison to that in negative control group ($P<0.01$, fig. 2).

![Fig. 1](image-url)