Study on the effect of integrin αvβ6 on the proliferation and apoptosis of thyroid carcinoma cells

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Summary

**Purpose:** To study the effect of integrin αvβ6 on the proliferation and apoptosis of thyroid carcinoma cells.

**Methods:** The experiment was conducted on 3 groups: the control group, the positive observation group (in which the αvβ6 on the surface of the thyroid carcinoma cell line SW579 was blocked by monoclonal antibody 10D5) and the negative observation group (in which the αvβ6 was dealt with the negative placebo of 10D5—the IgG2a). Cell proliferation was detected by MTT assay, apoptosis by flow cytometry and the protein levels in Caspase-3, CyclinB1 and Bcl-x1 as well as the protein levels in ERK, p-ERK, JNK, p-JNK, p38 and p-p38 were detected by Western blot.

**Results:** The cell survival rates of the control group and the negative observation group were prominently higher than those of the positive observation group, following decrease in the apoptosis rates, and the differences were statistically significant (p<0.05). The protein levels in CyclinB1 and Bcl-x1 of the control group and the negative observation group were prominently higher than those of the positive observation group, whereas the levels in Caspase-3 were decreased, the differences were statistically significant (p<0.05). The protein levels in p-ERK, p-JNK and p-p38 of the control group and the negative observation group were prominently higher than those of the positive observation group, while the protein levels of ERK, JNK and p38 showed no difference.

**Conclusion:** Integrin αvβ6 can mediate the MAPK signal pathway of the cells and regulate the expression of CyclinB1 and the apoptosis-related proteins like Bcl-x1 and Caspase-2, thus affecting the process of the proliferation and apoptosis of thyroid carcinoma cells.

**Key words:** apoptosis, integrin, MAPK signal transduction pathway, proliferation, thyroid carcinoma

Introduction

Thyroid carcinoma is the most common malignant tumor in the head and neck region with a high incidence and mortality rate among the neoplasms of the endocrine system. There are about 100,000 new cases in China every year [1], and surgical resection is the treatment of choice with recurrence and metastasis playing key roles on the survival outcome [2]. Integrin αvβ6, being a member of cell surface adhesion molecules family, mediates the interaction and bidirectional signal transduction between the cells and the extracellular matrix, and takes an important part in the adhesion, proliferation, differentiation, metastasis and apoptosis of cells [3]. It has been verified that αvβ6 is intimately correlated with the proliferation, apoptosis, grade of malignancy and prognosis of rectal and gastric carcinoma [4,5]. This study further analyzed the effect of αvβ6 on the proliferation and apoptosis of thyroid carcinoma and the mechanism of MAPK signal transduction pathway.
Methods

Experimental materials

Thyroid carcinoma cell line SW579 was purchased from Shanghai Biotech Co., Ltd, China. RPMI1640 cell medium and fetal calf serum were purchased from Beyotime Co., Ltd, Beijing, China. Mouse anti-human αvβ6 monoclonal antibody 10D5 and IgG2a were purchased from Sigma Corporation, St. Louis, MO, USA. Mouse anti Caspase-3, CyclinB1, Bcl-xl, ERK, p-ERK, JNK, p-JNK, p58 and p- p58 monoclonal antibody and mouse anti β-actin polyclonal antibody were purchased from Santa Cruz Corporation, CA, USA. MTT kit and DMSO were purchased from ZSGB-Bio Co., Ltd, Beijing, China. Annexin V-FITC/PI apoptotic kit and marker purchased from R&D Corporation, Minneapolis, USA were used for this study.

Cell culture and experiment grouping

The cell line was cultured in complete medium (RPMI 1640+10% fetal calf serum+1% penicillin-streptomycin) in the incubator under 5% CO2, saturation humidity and 37ºC. Cells in the logarithmic growth phase were taken in suspension and were inoculated on 96-well plate. Each well contained 1×10⁴ cells (100 μl volume). Three groups were established in this experiment: the control group (with no treatment), the positive group (in which the αvβ6 on the surface of the thyroid carcinoma cell line SW579 was blocked by monoclonal antibody 10D5) and the negative observation group (in which the αvβ6 was dealt with the negative placebo of 10D5—the IgG2a). After 30-min ice bath, the 3 groups were put into the incubator for another 6-hr culture, and the reaction was terminated by PBS wash.

Detection of cell proliferation

MTT assay was applied and the 96-well plate with 1×10⁴ cells in each well and 5 duplicate wells for each group were used. MTT was added until the concentration reached 5 mg/ml. After 4-hr incubation, 150 μl DMSO were added and the optical density value (OD) at 490 nm was detected by a microplate reader (Bio-Rad Corporation, Hercules, California, USA). The survival rate was estimated as: OD of the experiment group/OD of the control group×100.

Detection of cell apoptosis

The cells were resuspended to 1×10⁴ cells per well. After 10 min centrifuging at 2000g, the supernatant was discarded and 500 μl binding buffer was added. Then 5 μl AnnexinV-FITC and 10 μl propidium iodide (PI) were added. After 20 min incubation in the dark, a relevant detection and analysis were respectively performed by fluorescence activated cell sorting (FACS) caliber flow cytometry (BD Corporation, New Jersey, USA) and software WinMDI 2.9 (Applied Biosystems Corporation, City Foster, California, USA).

Detection of the apoptosis protein and signal pathway protein

Western blot method was used for protein detection. Proteins were extracted from the cells with conventional way (kit purchased from Invitrogen Corporation, Carlsbad, California, USA). Bicinchoninic acid (BCA) assay kit purchased from TaKaRa Corporation, Japan, was applied for the test of the protein concentration. Each sample in each well was loaded with 30 μg of protein, subjected to protein electrophoresis for 2.5 hrs at 36V (the electrophoresis apparatus was purchased from Beijing Liuyi Company), and transferred to PVDF membrane for 1.5 hrs at 60V (Costa Corporation, Corning, New York, USA). The samples were blocked with skimmed milk powder for 2 hrs, incubated with primary antibody (1:1000) at 4ºC overnight, and by second antibody (1:500) for 1 hr at room temperature. Enhanced chemiluminescence (ECL) was used for image development (Beijing Biotime Technology Co., Ltd), and Jieda 801 Analysis software 3.3 was applied for the OD detection of the stripe.

Statistics

SPSS20.0 software was applied for statistical analysis. The measurement data were presented as mean±standard deviation, one way ANOVA analysis was applied for comparisons among groups, and the LSD-t test was applied for comparison between groups. A p value <0.05 suggested that the difference was statistically significant.

Results

Comparison of cell proliferation

The cell survival rates of the control group and the negative observation group were prom-
Integrin αvβ6 and thyroid carcinoma cells

Comparison of cell apoptosis

Apoptosis rates of the control group and the negative observation group were prominently lower than those of the positive observation group, and the differences were statistically significant (p<0.05; Figure 2).

Comparison of the levels of apoptosis protein expression

The protein levels in CyclinB1 and Bcl-x1 of the control group and the negative observation group were prominently higher than those of the positive observation group, while the protein levels of ERK, JNK and p38 showed no difference (Table 2).

Discussion

Integrin αvβ6 is expressed only in embryogenesis, tissue repair and the epithelium-derived malignant tumors. Niu et al. [6] and Agrez et al. [7] have found that induction of overexpression of αvβ6 in colon cancer cells can stimulate the secretion of MMP-9 and degrade the extracellular matrix, which created a preferable mini environment for the proliferation and invasion of tumor cells, and was also the important occurrence mechanism of the hepatic metastasis and implantation of colon carcinoma. The proliferation of colon cancer cells can be promoted by αvβ6 through inhibition of the mitochondria apoptosis pathway [8]. Zhang et al. [9] have considered that the expression of αvβ6 in 36.7% gastric carcinoma patients, to be correlated with the tumor stage, lymphatic metastasis and recurrence and an individual risk factor of prognosis. The aforementioned results can be proved by specific antagonist of ανβ6, interference measure like RNA interference or antisense oligodeoxynucleotide technology and so on [10,11]. In the present study it was found that the cell survival rates of the control group and the negative

Table 1. Comparison of the levels of apoptosis protein expression

<table>
<thead>
<tr>
<th>Group</th>
<th>CyclinB1 (mean±SD)</th>
<th>Bcl-x1 (mean±SD)</th>
<th>Caspase-3 (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (mean±SD)</td>
<td>0.62±0.16</td>
<td>0.58±0.12</td>
<td>0.12±0.06</td>
</tr>
<tr>
<td>Negative observation group (mean±SD)</td>
<td>0.57±0.15</td>
<td>0.55±0.13</td>
<td>0.14±0.08</td>
</tr>
<tr>
<td>Positive observation group (mean±SD)</td>
<td>0.33±0.11</td>
<td>0.24±0.09</td>
<td>0.69±0.22</td>
</tr>
<tr>
<td>F</td>
<td>6.527</td>
<td>6.254</td>
<td>6.628</td>
</tr>
<tr>
<td>p</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the protein levels in the cell signaling pathways

<table>
<thead>
<tr>
<th>Group</th>
<th>ERK (mean±SD)</th>
<th>p-ERK (mean±SD)</th>
<th>JNK (mean±SD)</th>
<th>p-JNK (mean±SD)</th>
<th>p38 (mean±SD)</th>
<th>p-p38 (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (mean±SD)</td>
<td>0.52±0.16</td>
<td>0.55±0.12</td>
<td>0.43±0.13</td>
<td>0.29±0.09</td>
<td>0.65±0.21</td>
<td>0.44±0.15</td>
</tr>
<tr>
<td>Negative observation group (mean±SD)</td>
<td>0.53±0.17</td>
<td>0.32±0.14</td>
<td>0.44±0.12</td>
<td>0.26±0.08</td>
<td>0.65±0.22</td>
<td>0.42±0.17</td>
</tr>
<tr>
<td>Positive observation group (mean±SD)</td>
<td>0.54±0.18</td>
<td>0.14±0.07</td>
<td>0.42±0.15</td>
<td>0.08±0.03</td>
<td>0.64±0.23</td>
<td>0.19±0.08</td>
</tr>
<tr>
<td>F</td>
<td>0.126</td>
<td>5.627</td>
<td>0.154</td>
<td>5.218</td>
<td>0.096</td>
<td>5.459</td>
</tr>
<tr>
<td>p</td>
<td>0.867</td>
<td>0.000</td>
<td>0.823</td>
<td>0.000</td>
<td>0.921</td>
<td>0.000</td>
</tr>
</tbody>
</table>
observation group were clearly higher than those of the positive observation group, the apoptosis rate decreased, and the differences were statistically significant. It was noted that αvβ6 had similar effect on the biological behavior of thyroid carcinoma.

Caspase-3, a key factor in apoptosis, is generated in the terminal stage of all kinds of apoptotic pathways [12]. Mitochondrial apoptosis pathway is the principal internal apoptosis pathway of nucleated organisms, and the proteins of Bcl-2 family play a prominent role [13], including the anti-apoptosis members of Bcl-2 and Bcl-xL, which exert their effects on the outer mitochondrial membrane to maintain the integrity of membrane, as well as Bax and Bak which are functioning through destroying the integrity of mitochondrial membrane. Bcl-xL protein inhibits the release of cytochrome C through preventing the outer mitochondrial membrane from the destroying effect of Bax [14]; Bcl-xL can also form a ternary complex with Apaf-1 and Caspase-9 to realize the anti-apoptosis function [15]. Therefore, the change of the balance of pro-apoptosis and anti-apoptosis is a marking factor in the regulation process of mitochondrial apoptosis pathway. CyclinB1 is a significant cycle regulatory protein in the process of mitosis, it participates in the activity regulation of MPF and influences the activation and withdrawal of mitosis [16]. Research has proven that CyclinB1 protein level and subcellular localization vary with the cell cycle progression [17]. The level in G1 phase was extremely low, it gradually increased in S and G2 phases, and formed a MPF enzyme complex with CDK1, which was located in the centrosome, and of which the catalytic subunit CDK1 was phosphorylated to be deactivated. The protein level reached the peak in the late G2 phase, the N terminal being phosphorylated, which promoted the quick transduction import of MPF, and with the effect of CDC25C, the CDK1 region was phosphorylated and MPF activated. The mitosis-related proteins like lamin, histone and microtubulin were phosphorylated by the activated MPF, which led to karyotheca disintegration, chromosome agglutination and microtubule formation, and accelerated the process of cells entering the M period. In the medium and terminal phase of mitosis, CyclinB1 disappeared immediately through degradation, MPF deactivated, the chromatid separated, the cell mitosis terminated and the cells entered into the next cycle. This study showed that the protein levels in CyclinB1 and Bcl-xL of the control group and the negative observation group were clearly higher than those of the positive observation group, while the level in Caspase-3 decreased; the differences were statistically significant. It was noted that αvβ6 could regulate the expression of CyclinB1 and the apoptosis-related proteins like Bcl-xL and Caspase-2, thus affecting the process of the proliferation and apoptosis of thyroid carcinoma cells [18].

On one hand, the integrin connects with the extracellular matrix protein or adjacent cells, and the cytoskeleton system in the cell, thus forming the space link on which the survival of tumor cells relies. The integrin, on the other hand, acts as an acceptor to integrate with multiple cytokines, and through bilateral transduction of bio-information, it becomes an information hinge for the tumor cells to accept the outer stimulation and make reaction [19]. The αvβ6 can participate in several signal pathways like MAPK, TGF-β1, Fyn, PKC and so on, each being the cascade of the other one, and they connect with each other and exert synergistic effect [20,21]. Among those signal pathways, MAPK can activate multiple kinds of downstream proteins, and plays an important regulating role in cell proliferation, apoptosis and differentiation, etc. [22]. ERK is a significant part of the Ras-Raf-MEK-ERK cell signal transduction system which is the characteristic cascade in the MAPK signal transduction pathway, and also a key signaling molecule for the integrin to regulate the conversion, growth, invasion and metastasis of cells [23,24]. It was demonstrated in this study that the protein levels in p-ERK, p-JNK and p-p38 of the control group and the negative observation group were clearly higher than those of the positive observation group, while the levels in ERK, JNK and p38 showed no difference. It was noted that αvβ6 could regulate the proliferation and apoptosis of thyroid carcinoma cells through activation of the MAPK signal transduction pathway.

Conflict of interests

The authors declare no conflict of interests.
References