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Study on the expression protein of endoplasmic reticulum stress in the invasion and migration for tumor cells

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Abstract: **To study the effect and possible mechanism of endoplasmic reticulum stress for invasion and migration of the GIST cell. Using 10% fetal bovine serum to subculture GIST882 cell lines, using garment toxin to induce GIST882 for endoplasmic reticulum stress reaction, and then total protein were extracted and detected GRP78 and MMP-9 expressions using western blot. The healing method was used to detect the cell of migration ability and Trans well method was used to detect the cell of GRP78 cells of invasion ability. Compared with before induction, the expression levels of GRP78 protein after induced for 12h and 24h were significantly increased with time (P<0.05). Compared with the uninduced group, the cell migration and invasion ability in endoplasmic reticulum stress group were significantly enhanced, and the expression level of MMP protein was also significantly increased (P<0.05). Gastrointestinal stromal tumor has endoplasmic reticulum stress, and it may increase the expression of MMP–9 to promote cell invasion and migration.**

Keywords: Endoplasmic reticulum stress; Gastrointestinal stromal tumor; Attacks; Migration

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1. Introduction

Studies suggest that endoplasmic reticulum stress plays an important role in tumor development, and it is widespread in variety of solid human tumors, and it is a positive correlation with tumor infiltration depth, migration and transfer capability. Endoplasmic reticulum stress is the important mechanism of the tumor invasion and metastasis[1]. Domestic studies also suggest that it exists in breast cancer, liver cancer, and pancreatic cancer. As cells' self-protection reaction, is closely related to the malignant biological behavior of tumors progress[2,3]. However, about the relations of endoplasmic reticulum stress and gastrointestinal stromal tumor cell invasion and metastasis and the mechanism, it is unclear. Therefore, cytology biological technology is adopted in this study to detect the endoplasmic reticulum stress response proteins and gastrointestinal stromal tumor cell invasion and migration ability.

2. Materials and Methods

2.1. Cell culture

GIST 882 cells were conventional recovered from Sun Yat-sen University. Cell were cultured with 10% fetal bovine serum culture medium for 0.05mg/ml of 1640 medium, and culture at 37℃ constant temperature box. CO_2 concentration was 5%. When the cell grows to the bottom of the bottle, it is digested and passed on.

2.2. Endoplasmic reticulum stress cell model

Endoplasmic reticulum stress cell model were made with garment. After being stable cell growth, the logarithmic phase GIST 882 cells were digested with 0.25% trypsin. The cell suspension were planted to three medium, and three groups of cells were added 5ug/ml garment, continue to culture for 12h, 24h and 36h. Control group was added the same amount of liquid culture medium only.

2.3. Western blot

Digest and collect each group cell, add protein cracking to extract total protein. The protein concentration determinate. The sample were added to the fluid of the electrophoresis for transfer film, using 5% skimmed milk powder fully closed PVDF membrane. PVDF membranes were cultured for 12 hours with first resistance in low temperature. Flush for 3 times with buffer, and then plused second resistance to incubate for 4 hours in the sealing machine. ECL luminous fluid was used for exposure.

2.4. Migration experiment in vitro

The GIST 882 cells were inoculated in 6 orifice, continue to cultivate to 95% full of culture plate, using sterile pipetting spear to scratch the cells, after scratch, rinsing off cells and to join the media continue to develop, to observe the cells after cultivating for 0, 12, 24 and 36 h to observe the cell growth.

2.5. Transwell invasion experiment

The logarithmic phase cell suspension were snapped to transwell little room by 100μL (2×10^5m/L). The proportion of vaccination in the up room, and to add 600μl sugar medium incubation including fetal bovine serum for 24h. After 24h, under room were taken out

and fixated and stained using form aldehyde.

2.6. Statistical processing

Using SPSS19.0 statistical software, the quantitative data of each group were analyzed, and the variance analysis was adopted, and the q test was further used. And the difference with P<0.05 was significant.

Table 1. Expression of MMP-9 and GRP78 protein before and after endoplasmic reticulum stress

Times	GRP78expression	MMP-9expression
0	0.336±0.022	0.221±0.032
12h	0.974±0.054	0.621±0.021
24h	1.147±0.046	0.716±0.035
36h	1.254±0.078	0.847±0.064
F	4.332	5.210
P	0.042	0.021

3. Results

3.1. Expression of MMP-9 and GRP78 protein before and after endoplasmic reticulum stress

The expression levels of GRP78 protein in 12h and 24h were significantly increased than before, and with significant time dependence, and the difference was statistically significant (P<0.05), as shown in Figure 1, Table 1.

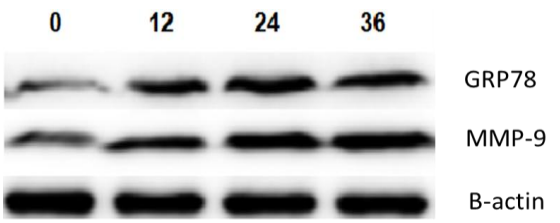

Figure 1. Expression of MMP-9 and GRP78 protein before and after endoplasmic reticulum stress.

Table 2. Changes of migration capacity for GIST882 cells before and after induction

Times	Not induction group	Induction group
12h	14.94±4.14	21.62±3.65
24h	21.62±5.33	30.25±5.12
36h	28.17±5.71	38.77±6.65
F	6.339	8.147
P	0.001	0.001

3.2. Changes of migration capacity for GIST882 cells before and after induction

Compared with the uninduced group, the healing rates of 12h and 24h and 36h cells were (21.62±3.65)%, (30.25±5.12)% and (38.77±6.65) %, respectively, (P < 0.05), as shown in Table 2.

3.3. Changes of cell invasion ability before and after induction

Trans well method showed that cell invasion ability was significantly increased, and the comparison between groups was statistically significant (P < 0.05), as shown in Table 3, Figure 2.

Table 3. changes in the invasion ability of the post-gist882 cells before and after induction

Times	Not induction group	Induction group
12h	32±4.1	44±5.2
24h	50±5.3	78±8.1
36h	68±6.7	105±14.7
F	5.996	12.36
P	0.001	0.001

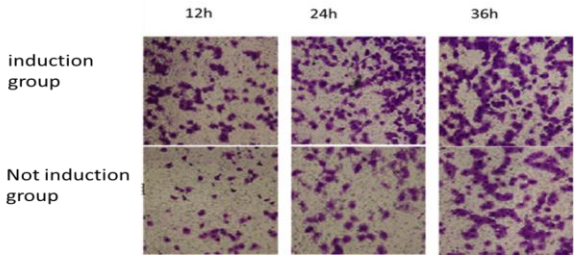

Figure 2. Changes of cell invasion ability before and after induction.

4. Discussion

The gastrointestinal stromal tumor is related to many factors. Endoplasmic reticulum stress is considered as another important mechanism for the development of tumor. Clinical studies have found that gastrointestinal stromal tumor is not sensitive to traditional chemotherapy regimens, but it has high curative effect for imatinib mesylate, its principle may be as targeted drugs for tumor cell signaling pathways protein of c kit or PDGFRα[4]. But this kind of drug is very expensive, and also appear drug resistant. Therefore, it needs to find new therapeutic targets and guarantees for clinical treatment of the tumor. The endoplasmic reticulum stress is generally exists in tumor cells as a protective reaction. But endoplasmic reticulum stress pathway proteins can or not get more curative effect as targets for clinical.

Endoplasmic reticulum stress proteins of GRP78 are an important member of the molecular chaperone, were highly expressed in the groups of solid tumors. When tumor cells occurs endoplasmic reticulum stress, GRP78 level increased obviously, but when under the not stress condition, GRP78 combined with endoplasmic reticulum trans-membrane protein, and being in the inactive state. Therefore, the high expression of GRP78 is considered to detect the existence of endoplasmic reticulum stress in the cell of

the gold standard[5-8]. This research adopts gastrointestinal stromal tumor cells endoplasmic reticulum stress model, using garment genetically altered its principle is by inhibiting cell glycoprotein synthesis of N-sugar chain so as to induce the occurrence of endoplasmic reticulum stress[8-11]. Study found that compared with before induction, after 12h, 24h, GRP78 protein level in induction cell increased significantly, and has obvious time dependence. It shows that the strength of the cells of endoplasmic reticulum stress increased. With prolonged garment toxin, it can be successfully established the endoplasmic reticulum stress gastrointestinal stromal tumor model, in order to further study the endoplasmic reticulum stress and tumor cell invasion and the relationship .

Walte[12] research shows, with the exception of tumor, tumor cells active hyperplasia caused by tumor proliferation, and high-speed proliferation needs nutrition support. Therefore, when not enough oxygen to tumor cells, cells were in hypoxia state, and oxygen is powerful inducers for endoplasmic reticulum stress. Endoplasmic reticulum stress is common in malignant tumor cells and in order to adapt to the change of micro environment, by activating protein folding reaction, enhancing growth factor, cell factor and angiogenesis factor, tumor cells had to adjust the tumor micro environment, thus promotes cell migration and metastasis of tumor[13-15]. At present, the study found the MMP-9 has the closest relations with tumor migration[16]. Our study process GIST882 cells with garment toxin for 12h, 24h and 36h. As the growth of the time, migration and invasion of cells obviously increase and present time dependence. Western blot results show that the MMP-9 protein levels also increased significantly.

5. Conclusion

The results showed that the endoplasmic reticulum stress cells by adjusting the MMP-9 to promote the occurrence of the migration and metastasis, but the more specific reason need to be further in depth study. But the result of this study confirmed that the endoplasmic reticulum stress occur in gastrointestinal stromal tumor, and endoplasmic reticulum stress can increase the expression of MMP-9 of gastrointestinal stromal tumor cells, and promote cell invasion and migration.

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