RESEARCH COMMUNICATION

Effects of Monoclonal Antibodies against Human Stathmin Combined with Paclitaxel on Proliferation of the QG-56 Human Lung Carcinoma Cell Line

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Abstract

Objective: To explore whether monoclonal antibodies against stathmin and the chemotherapeutic agent paclitaxel have synergistic effects in inhibiting growth and inducing apoptosis in human QG-56 cells. Methods: QG-56 cells were treated with monoclonal antibodies against stathmin or paclitaxel alone or in combination, with untreated cells used as controls. After 24, 48, 72 and 96 hours the cell growth condition was observed under an inverted microscope and inhibition was studied by MTT assay; apoptosis was analyzed by flow cytometry. Results: The populations decreased and cell shape and size changed after the various treatments. Monoclonal antibodies against stathmin and paclitaxel used alone or in combination inhibited the proliferation of QG-56 cells, especially in combination with synergism (P<0.05). Combined treatment also resulted in a significantly higher apoptosis rate than in the other groups (P<0.05). Conclusions: Monoclonal antibodies against stathmin and paclitaxel used alone or in combination can inhibit proliferation of QG-56 cells and induce apoptosis when applied together. The observed synergistic effects may have important implications for clinical application.

Keywords: Monoclonal antibodies against stathmin - paclitaxel - lung carcinoma - proliferation - apoptosis

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Introduction

Lung cancer is one of the most harmful malignant tumors to human health and life, and its incidence is increasing every year, and ranking the first in large and medium-sized cities in the incidence of malignant tumors. 2/3 of patients are in advanced stage when diagnosed as lung cancer and lose the opportunity of surgical treatment. Chemotherapy is primarily used for the treatment of advanced lung cancer. Although new anti-cancer drugs and chemotherapies have been introduced, the outcomes for some patient are not satisfactory, and patients are becoming less tolerated as the chemotherapy extends. Therefore, targeted therapy of lung cancer becomes the research hotspot in recent years. Recently, erlotinib and gefitinib are widely used in lung cancer therapy, but both of which are only effective for specific pathological situations and patients, the outcomes for rest of the patients are still not satisfactory (Zhang et al., 2005; Zhou et al., 2008).

Stathmin is a newly discovered target for gene therapy in cancer in recent years. Stathmin is a soluble protein which plays a very important role during cell proliferation and differentiation, 20kD, consisting of 149 amino acid. Studies of cell signal transduction shows that a variety of cytokines, protein product of oncogenes and tumor suppressor genes from inside and outside of cells could lead to cellular changes either directly or indirectly. Stathmin is the substrate of a variety of intracellular kinases, and its downstream targets includes tubulin, microtubules, spindle and other organelles playing a key role in cell cycle. By adjusting the balance of microtubule dynamics, stathmin could control the cell cycle, and thus change the biological behavior of cell proliferation, differentiation, and activity (Segmnan et al., 2003; Rubin and Atweh, 2004). The high level of stathmin expression is detected in a variety of malignant tumors in breast, lung, leukemia, etc. And the repression of stathmin expression could disturb the division of malignant cells and promote the reversal of malignant phenotype of tumor cells (Mistry and Atweh, 2002; Hsieh et al., 2010; Wang et al., 2010; Wang et al., 2010). Paclitaxel is recognized as a broad-spectrum anti-cancer drug with strong activity and unique anti-cancer mechanism. It is proved that paclitaxel is efficient in a variety of cancers, especially lung cancer, ovarian cancer and breast cancer treatment, the rate of which could be up to 75% (Flores et al., 2011; Le et al., 2011).

In this study, we treated the human lung carcinoma QG-56 cells with paclitaxel and stathmin monoclonal antibody for human, and tested the efficiency of inhibition, provide the basis for further in vivo testing and clinical application.

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Materials and Methods

Cells and reagents

Human lung cancer cell line QG-56 passaged and preserved in Institute of antibody engineering in Southern Medical University. Paclitaxel were purchased from Beijing Hua Su Pharmaceutical Co., Ltd. Stathmin monoclonal antibody is produced in, Institute of antibody engineering, Southern Medical University. RPMI-1640 medium, 10% newborn calf serum, double-antibody containing 100 μg/ml penicillin and 100 μg/ml streptomycin, and 0.25% trypsin solution were purchased from Gibco. Thiazolyl tetrazolium (MTT), analytical grade dimethyl sulfoxide (DMSO) were purchased from Sigma Corporation. Annexin V/PI double staining kit was purchased from Shenzhen Jingmei Company. CO₂ incubator was made in Thermo. Inverted phase contrast microscope was purchased from Olympus Company in Japan, an automatic microplate reader from Anthos Company and a flow cytometer from Becton Dickinson.

Morphological changes of QG-56 cells

Take the QG-56 cells in logarithmic phase, and inoculate them on six pore plate 2 × 10⁵/pore. 4 hours later, add stathmin monoclonal antibody with different concentrations (10, 20, 40, 80 and 160 μg/ml) and paclitaxel with different concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 μg/ml), and a combination of paclitaxel and stathmin monoclonal antibody. Morphological changes were recorded with inverted phase contrast microscope after 24, 48, 72 and 96 hours of treatment.

Detect the inhibition of QG-56 cells proliferation with MTT assay

Take the QG-56 cells in logarithmic phase, and inoculate them on 96 pore plate 2 × 10⁵/pore, cultured in 37 °C, 5% CO₂ for 4 hours. Then add drugs with different concentrations to each pore (the same as 1.2), with another group without drugs as a control, each dose with 5 additional pores. Add 20 μl MTT (5 mg/ml) to each pore after cultured for 24, 48, 72 and 96 hours and culture for another 4 hours. Then remove supernatant, add 150 μl DMSO to each well, oscillate for 10 min to fully melt the crystals and detect the of the absorbance value (A). Set the cellular viability of control group as 100% and calculate the inhibition rate of each group. The inhibition rate (%) = (A490 value of control group - A490 value of the treat group) / A490 value of control group × 100%.

Detect the apoptosis of QG-56 cells with flow cytometry

Take the QG-56 cells in logarithmic phase, and inoculate them on six pore plate 2 × 10⁵/pore. Then add drugs with different concentrations to each pore (the same as 1.2), with another group without drugs as a control, each dose with 4 additional pores. Collect samples with trypsin, fix with 75% (volume fraction) ethanol overnight under 4 °C and wash them 2 times with PBS. Digest the samples with RNase A (1 mg/ml) for 30 min under and stain with propidium iodinate (100 ug/ml) for 20 min under 4 °C. Separate 10⁶ cells for each group of samples and process the data with ModFit T V2.0.

Results

The number and morphology of MCF-7 cells is basically the same before the treatment between control group and experimental group. 24, 48, 72 and 96 hours after treatment, cells in control group grow well, and the cell number increases as the cells grow for a longer time. The cell sizes and morphology is uniform in control group. And loose chromatin and abundant cytoplasm is observed under high power lens. In the experimental group, after cultured for 48 hours, the cell number decreases in each sample. The cells are different in sizes with irregular morphology. Under high power lens, we observed nucleus condensation, cytoplasmic reduction and vacuoles in the cytoplasm. And some cells are off the wall and in semi-suspended state. The number of cells and morphological changes is positively correlated with the duration of drug treating and concentration (Figure 1).

The combination of McAb and paclitaxel shows also time and concentration-dependent (Table 2).
Stathmin is also known as Opl8, p19 and Metablastin which is the product of lymphoma-specific gene. It consists of 149 amino acid whose molecular weight is 20 kD. It is ubiquitous in vertebrate cells and a highly conserved cytoplasmic phosphoprotein. Stathmin has two functional domains: one is the “adjustment” domain in N-terminal. The 16 \text{th}, 38 \text{th} and 63 \text{th} amino acid of the domain is serine (Ser16, Ser25, Ser38 and Ser63), which are the target sites of protein kinase mediating the phosphorylation of stathrin. The other is “effect” domain in C-terminal. There is a spiral structure which interacts with other proteins in the form of a helix-helix domain. Studies show that the stathmin expression of 8 domains: one is the “adjustment” domain in N-terminal.

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Discussion
Stathmin is also known as Opl8, p19 and Metablastin which is the product of lymphoma-specific gene. It consists of 149 amino acid whose molecular weight is 20 kD. It is ubiquitous in vertebrate cells and a highly conserved cytoplasmic phosphoprotein. Stathmin has two functional domains: one is the “adjustment” domain in N-terminal. The 16, 25, 38 and 63 amino acid of the domain is serine (Ser16, Ser25, Ser38 and Ser63), which are the target sites of protein kinase mediating the phosphorylation of stathmin. The other is “effect” domain in C-terminal. There is a spiral structure which interacts with other proteins in the form of a helix-helix domain. Studies show that the variety of cytokines, protein product of oncogenes and tumor suppressor genes from inside and outside of cells could lead to cellular changes either directly or indirectly (Jen et al., 2010; Yu et al., 2010; Zheng et al., 2010). The high level of stathmin expression is detected in a variety of malignant tumors such as ovarian cancer, lung cancer, liver cancer, osteoblastoma, breast cancer, etc. Researchers speculate that the commonly observed high expression of stathmin in tumor cells is merely a reflection
of enhanced proliferation activities of tumor cells. Subsequent studies have confirmed that the high level of stathmin expression is required for the maintenance of high proliferation rate of tumor cells. Drugs that could inhibit cell division through preventing microtubule have been widely used in tumor chemotherapy. Studies confirmed that stathmin overexpression is associated with the sensitivity of lung cancer cells to vindesine, which could be used as a marker of vinca alkaloids-sensitive in lung cancer patients. The combination of paclitaxel and anti-stathmin provides an effective, low toxicity treatment, which is much safer than the combination of multiple chemotherapy drugs that may cause the toxicity accumulation (Alli et al., 2002; Balachandran et al., 2003).

The stathmin gene therapy is receiving more and more success in experiments, which could be confirmed via inhibiting its expression through the antisense oligonucleotide, ribozyme, stathmin protein inhibitors, mutating serine sites and other strategies. The repression of stathmin expression could make the cell cycle blocked in G2/M phase and reverse the phenotype of the malignant tumors (Le et al., 2010). But there are little researches about gene therapy on the monoclonal antibody of stathmin. Monoclonal antibody drugs study is very active in the biopharmaceutical field. The monoclonal antibody is highly specific for a specific molecular target (antigen). A variety of monoclonal antibodies could be produced for different antigens. So, as a drug source, the monoclonal antibody has a high degree of diversity. Because of its specificity and diversity, there are great potentials for the development of monoclonal antibody drugs. To date, many of the targeting drugs have played an extremely important role in clinical. According to the principles of evidence-based medicine, some of these drugs have been recorded in standard treatment protocols and norms recognized by the international oncology community (Tuschl and Borkhardt, 2002; Hainsworth et al., 2004; Lin et al., 2009; Su et al., 2009; Xi et al., 2009). We successfully prepared the anti-stathmin monoclonal antibody, and lay the foundation for this experiment. Paclitaxel is a kind of novel natural anticancer drugs with unique structure and mechanism, which is extracted from the bark and trunk of the taxus. It is also currently the only one novel anti-tumor drug with unique anti-microtubule mechanisms and broad-spectrum effect. Paclitaxel represses tumor through the synthesis of microtubule. And in particular, it promotes the formation of stable microtubule polymers and inhibits their depolymerization, so the number of free microtubules decreased significantly, which inhibits the separation of the spindle toward the poles, further inhibiting mitosis. Paclitaxel could also make cell cycle blocked at G2/M phase and induce apoptosis.

In this study, we tested the effects of anti-stathmin monoclonal antibodies, paclitaxel and their on the lung cancer cell proliferation via MTT assay. The results show that both either of them alone and combination of them could both significantly inhibited QG-56 lung cancer cell proliferations. And the inhibition intensity increases with the increase of drug concentration and treatment duration, showing a certain amount of time a dose dependent manner. The combination of anti-stathmin monoclonal antibodies and paclitaxel showed greater inhibition than either of them alone, indicating obvious synergies between the two drugs. Apoptosis assay showed that the changes of the apoptosis rate also dependent on time and dose. The combined use of anti-stathmin monoclonal antibody and chemotherapy drugs have a good prospect, lay a foundation for further in vivo testing and will provide new ideas for clinical treatment.

References


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