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Original Article

Impact of sex hormone on small cell lung carcinoma xenograft model in nude mouse

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Abstract: Recent research found that the abnormal secretion of estrogen and its receptor play important roles in pathogenesis of small cell lung carcinoma (SCLC). But there are few studies investigating the effects of sex hormone on the pathogenesis of SCLC. Our study aims to investigate the effects of different sex hormone on the SCLC xenograft model in nude mouse. A SCLC cell line, MMSCLX-07, was stimulated with different concentration of sex hormone. The MTT assay was used to measure the relative vigor of cells to choose the optimum stimulating concentration. Total of 36 BALB/c-nu nude mice were divided into 3 experimental groups and were respectively inoculated suspension of MSCLX-07 cells which were stimulated with different sex hormone. Every nude mouse was inoculated for two points. Long radius and short radius of xenografted tumors were measured and growth curves were drawn. Forty-five days later, the HE staining was performed to observe the growth of xenografted tumor tissues. The optimum concentration for stimulating MMSCLX-07 cells was 10 nM hormones. The tumor formation rate in 3 groups was 0, 8.3% and 75%, respectively. There was significant difference between androgen and estrogen stimulation group ($P<0.01$), there was no significant difference between androgen stimulation and normal saline control group ($P>0.05$). The results of HE staining of xenografted tumor tissues showed that the tissues stimulated by estrogen were SCLC. Under the stimulation of 17β-estradiol, MMSCLX-07 cells could induce development of xenografted tumor in nude mice. Therefore, the Estrogen may play an important role in the pathogenesis of SCLC.

Keywords: Estrogen, small cell lung cancer, nude mouse, tumor-bearing experiment

Introduction

The incidence of Lung cancer dominants the highest in China. Of lung cancer, the incidence of small cell lung carcinoma (SCLC) accounts about 20%, which is clinically characterized by quick proliferation, high invasion, and easy metastasis in early stage, and sensitivity to chemotherapy but easy occurrence of drug resistance [1-3]. The previous studies suggest that SCLC originate from bronchial epithelium which could divide into stem cells with neuroendocrine [4-7]. And cytoplasm of tumor cells have typical axis neuroendocrine granular [5]. The immunohistochemical results showed that the tumor cells are reactive positively to the NSE, 5-HT, CgA under electron microscope, which proved that the SCLC with the properties of neuroendocrine function [8-10]. Current study suggested that pathogenesis of female tumor is associated with estrogen level with abnormal expression of estrogen receptor. Recent researches found that the abnormal secretion of estrogen and its receptor play an important role in pathogenesis of SCLC [11-16]. Estrogen receptors are widely distributed in SCLC tissues. But there are few studies investigating the effect of the sex hormone on the pathogenesis of SCLC.

Small cell lung carcinoma (SCLC) was mainly characterized by the lower differentiation, higher malignancy and earlier metastasis, which is also a serious threat to the public health. Some studies have confirmed that the SCLC possesses the property of neuroendocrine function and estrogen receptors are widely distributed in SCLC tissues. But there are few studies on effect of the sex hormone on the pathogenesis of SCLC. Therefore, after stimulating SCLC cells

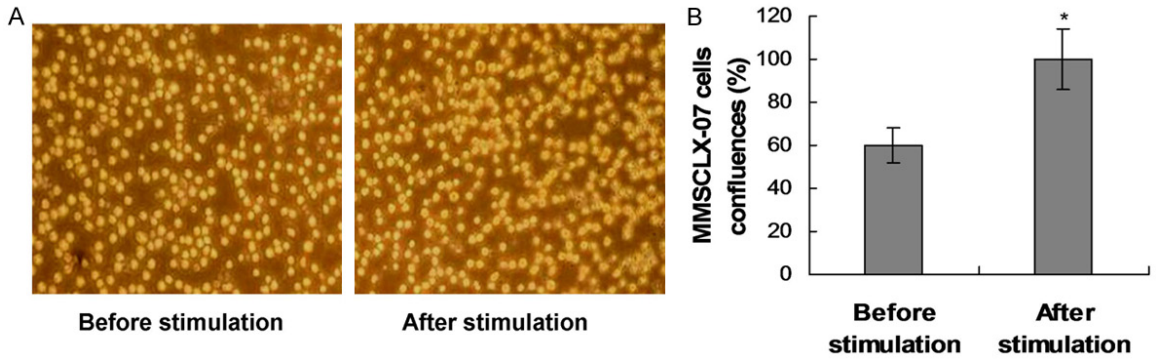

Figure 1. 24 hours after stimulation with 17β-estradiol, MMSCLX-07 cells confluences were increased from 60% to 100%. A. MMSCLX-01 cells observed by using the microscope. B. Statistical analysis. *P<0.05 represents the cells confluences after stimulation compared to before stimulation.

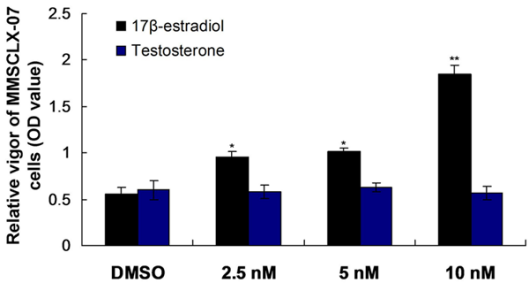

Figure 2. Vigor (or cell viability) of MMSCLX-07 cells under stimulation of different concentrations of sex hormone. Vigor was the best after dealt with 10 nM 17β-estradiol. *P<0.05, **P<0.01 represent the relative vigor of MMSCLX-07 cells in 17β-estradiol group compared to the testosterone group.

(MMSCLX-07) with sex hormone, these cells were inoculated to BALB/c-nu mouse to copy small cell lung carcinoma xenograft model in nude mouse. Tissues of xenografted tumors were selected and underwent the HE staining to observe the growth of tissues of xenografted tumor and evaluate the effect of sex hormone on the pathogenesis of SCLC, which will provide the experiment foundation for the further researches.

Materials and methods

Experimental cells and animals

Experimental cells: SCLC cells (MMSCLX-07) were provided by Southern Medical University. Cell culture medium of RPMI-1640 which contained 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/m streptomycin was added and cultivated at 37°C in CO_2 incubator. About 3 to 4 days later when cells filled in cultivated bottle, cells were underwent 1:4 passages. Cells used in our study were cells in logarithmic growth.

Experimental animals: Total of 36 female BALB/c-nu nude mice with SPF grade, weighted (15±3) g at 4 weeks of age, were brought from Beijing Mellier experimental animals technology Co,. Ltd. One week before experiment, the nude mice were acclimatized in the feeding environment at room temperature of (22±3)°C and humidity of 35% to 70% to accommodation.

MMSCLX-07 cells stimulation with sex hormone

The 4×10^3 MMSCLX-07 cells in logarithmic growth were implanted into 96 pore plates and put into 1640 cell culture medium of without phenol red dealt with 10% activated carbon after 24 hours. Corresponding concentration of testosterone and 17β-estradiol were added to make a concentration gradient of 2.5 nM, 5 nM, 10 nM and were cultured continuously at 37°C in 5% CO_2 incubator. About 48 hours later, 20 μL MTT of 5 mg/ml were added at 37°C in 5% CO_2 incubator for reacting for 4 hours. Culture solutions were then abandoned. 100 μL DMSO was added to cease reaction. Multimode reader of 570 nm was used to measure OD value.

Establishment of tumor-bearing mouse

According to the experimental results of cells stimulation with sex hormone, MSCLX-07 cells after stimulated with 10 nM sex hormone were used to the subsequent experiment. Thirty-six

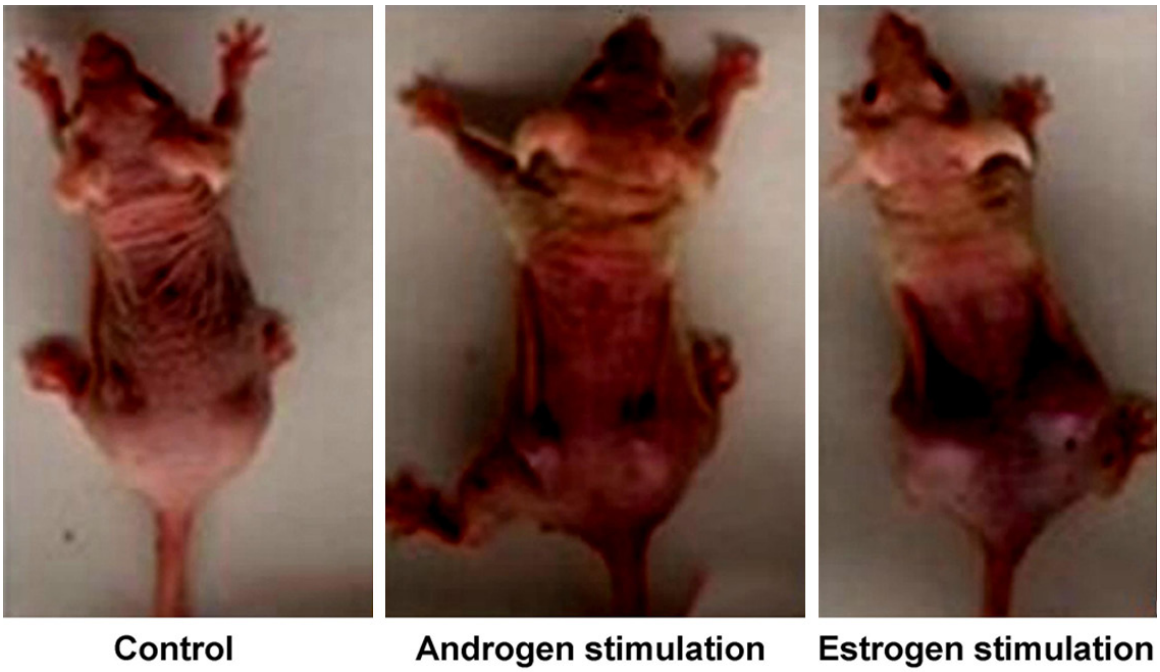

Figure 3. There was no tumor formation in 12 nude mice of control group. One of the 12 nude mice in androgen stimulation group had tumor formation. And 9 of the 12 nude mice in estrogen stimulation group had tumor formation.

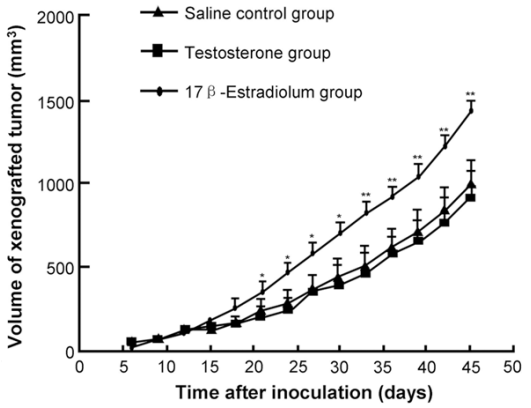

Figure 4. Growth curve of xenografted tumor in nude mice of each group. Deaths in each group: There were 3 deaths of nude mice at 14, 18 and 21 days after inoculation and 1 death at 43 days in estrogen group. There was no death in androgen group. *P<0.05, **P<0.01 represent the volume of xenografted tumor in 17β-estradiol group compared to the testosterone group.

nude mice were divided randomly into three groups, including normal saline control group (12 mice), androgen stimulation group (12 mice) and estrogen stimulation group (12 mice). 10 nM MSCLX-07 cells of 48 hours after stimulated with sex hormone were adjusted to make a concentration of 1×10^7/ml, 0.2 ml cell suspensions were injected subcutaneously into left and right forelimb interior of nude mice respectively. About 7 days later, through visual inspection of subcutaneous tissue of nude mice, if there are growing tumor-like tissues, SCLC nude mouse model is successfully established. After inoculation, experimental nude mice were under observation for spirit, respiratory, movement and tumor growth in the legs and weighted early day. The related data was recorded in each experiment.

HE staining of xenografted tumor tissues in nude mice

Xenografted tumor volume in nude mice was calculated according to the spheroid volume formulation {V=π/6× (A/2+_B/2) 3}. Nude mice with xenografted tumor volume of 400~600 mm^3 were screened for subsequent experiment. At 45 days nude mice were executed by routine methods. Xenografted tumor tissues in nude mice were fixed with 4% paraformaldehyde and embedded by paraffin. The 6-μm-thick sections were prepared and the slides were performed by using the HE staining. Xenografted tumor tissues in nude mice were observed for pathological changes.

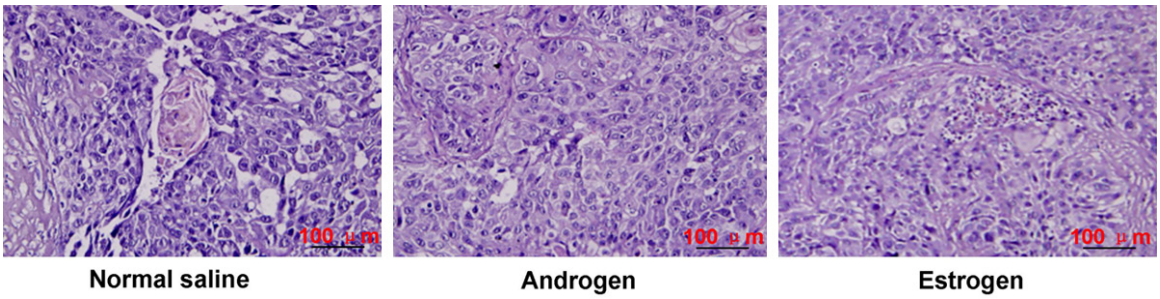

Figure 5. HE staining of normal saline control, androgen stimulated and estrogen stimulated groups. The scale bars have been added as 100 μm.

Statistical analysis

We performed all analyses by use of SPSS Software version 19.0. Numerical variables were reported in terms of mean and standard deviation ($\bar{X}\pm s$) and were analyzed between the 2 groups by using the Student t-test. Chi-square test was used to analyze comparison of independent sample of multiple groups. Variables showing *P*-value less than 0.05 were considered to be statistically significant difference.

Results

Growth of MMSCLX-07 cells after stimulation with sex hormone

Through observation by inverted optical microscope, 24 hours after stimulation with testosterone, MMSCLX-07 cells stopped growing with no change of morphology. 24 hours after stimulation with 17β-estradiol, the MMSCLX-07 cells had normal growth, cell confluence was increased from 60% to 100% (**Figure 1**). The results of relative vigor of MMSCLX-07 cells measured by MTT methods showed that vigor of MMSCLX-07 cells was the best after dealt with 10 nM 17β-estradiol, which indicated that stimulation with concentration of 10 nM was the most effective (**Figure 2**).

Tumor formation of xenografted tumor

After inoculation, there was no tumor formation in 12 nude mice in control group. One of the 12 nude mice in androgen stimulation group with the obvious tumor formation. And 9 of the 12 nude mice in estrogen stimulation group also with obvious tumor formation. The tumor formation rate in 3 groups was 0, 8.3% and 75%, respectively. Through χ^2 test we found that there was significant differences between androgen and estrogen stimulation group ($P<0.01$), and there was no significant differences between androgen stimulation and normal saline control group ($P>0.05$) (**Figure 3**).

Growth of xenografted tumor

Volume of tumor was treated as Y-axis and observation time was treated as X-axis and growth curve of xenografted tumor was drawn (**Figure 4**). The growth rate of xenografted tumor in estrogen stimulation was significantly higher compared to the androgen stimulation group and normal saline group ($P<0.01$). When calculating the volume of tumor, xenografted tumor in estrogen stimulation had equal size and little mutation.

HE staining of xenografted tumor in nude mice

Compared with the HE staining of normal saline control group and androgen stimulation group, the xenografted tumor cells in estrogen stimulation group also illustrated the significant properties of SCLC, which were exiguity of cytoplasm, obscure boundary, fine granular of nuclear chromatin, and no or indefinite nucleolus, in round, oval or fusiform shape, with clear nuclear incisures, typical necrosis with universality and with high count of karyokinesis (**Figure 5**).

Discussion

Early diagnosing SCLC is difficult. Therefore, when it was found to be symptomatic, most of patients have metastasis and the SCLC is a great thread to public health. Some of the studies have found that over estrogen levels are associated with poor prognosis of male and female lung cancer. And Vermam K *et al.* found

that high expression of estrogen β receptor in cytoplasm was the important biomarker for detecting SCLC [17]. Therefore, we assume that estrogen may play an important role in the pathogenesis of SCLC. In the present study, MMSCLX-07 cells were stimulated by androgen and estrogen to make xenografted tumor in nude mice. We found that tumor formation rate after stimulating MMSCLX-07 cells with 10 nM sex hormone was significantly higher than the stimulating MMSCLX-07 cells with normal saline and androgen. The above results indicated that estrogen may play an important role in the pathogenesis of SCLC.

SCLC is pathologically classified as SCLC and mixed lung cancer (small cell cancer and mixed type of squamous cell carcinoma or adenocarcinoma) [18-20]. It was histologically characterized by exiguity of cytoplasm, obscure boundary, fine granular of nuclear chromatin, no or indefinite nucleolus, in round, oval or fusiform shape, with clear nuclear incisures, typical necrosis with universality and with high count of karyokinesis [21, 22]. In present study, HE staining results of xenografted tumor stimulated by estrogen was consistent with the histological characteristic of SCLC. These results indicated that estrogen could induce formation of SCLC in nude mice and played an important role in pathogenesis of SCLC.

According to the results of the present study, we suppose that SCLC may be a sex hormone dependent tumor. Therefore, treatment of SCLC should take into consideration for the effects of estrogen. Our study provide a new promising insight investigating pathogenesis and treatment of SCLC, which will provide an experiment foundation for the further researches.

Disclosure of conflict of interest

None.

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