Study on Functional Monoclonal Antibodies of Anti-Human Uterine Sarcoma Stem Cell-Like Cells

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Abstract

**Background:** To establish a functional monoclonal antibody library using Human Uterine Sarcoma Stem Cell-Like Cells (HUSSLCs) to screen and identify functional monoclonal antibodies that can recognize and inhibit HUSSLCs.

**Methods:** B lymphocytes in proliferative state were prepared by using the second generation CD133+spheroid cells of SK-UT-1 cell line, i.e. HUSSLCs, as antigens; Spheroid formation, agar colony formation, wound healing, flow cytometry, and Western blotting were adopted to detect the effect of monoclonal antibodies with varied dilution ratios on HUSSLCs spheroid formation, agar colony formation, cell migration, CD133 expression, and expression of CD44, ABCG2, Bmi1, Nanog, Oct4 and ALDH1.

**Results:** Myeloma cells of SP2/0 cell line can achieve 85% degrees of fusion and results of 1-2F monoclonal cell supernatants with different dilution ratios reduced HUSSLCs spheroid formation rate, agar colony formation rate, cell migration rate, CD133 positive cell expression and protein expression levels of CD44, ABCG2, Bmi1, Nanog, Oct4, and ALDH1 in concentration-dependent manner (P <0.05).

**Conclusion:** The antibody valence produced by HUSSLCs-immunized mice reached the requirement for preparation of monoclonal antibody. Anti-HUSSLCs monoclonal antibodies feature functions of inhibiting the self-renewal, unrestricted proliferation, migration, invasion and multidrug resistance of HUSSLCs and functions characterized by tumor stem cells.

**Background**

Among all malignancies of the female genital system, uterine sarcoma is rare, the incidence of which only accounts for 2-4% of uterine malignancies, but with high degree of malignancy, poor prognosis, and a 5-year survival rate of only 30%-50%, contributing to
25% of mortality induced by uterine malignancy. One key reason causing this phenomenon lies in that most uterine sarcomas are insensitive to radiotherapy and chemotherapy, coupled with strong invasion and metastasis ability, as well as easy relapse. The limited treatment for uterine sarcoma and the high mortality rate corresponding to its low incidence rate make the search for a new and effective treatment method is becoming an urgent need for obstetricians and gynecologists.

Stem cells are a special type of cells in the body that boast significant biological properties of both self-renewing and multi-directional differentiation. Studies found that the degree of malignancy rises with the content of TSCs in tumor cells. However, current research evidence still not yet suffice enough to prove the existence of TSCs in all tumor tissues. TSCs play an essential role in the overall development of tumors and are more genetically unstable and more able to adapt and survive in new environments compared with common tumor cells. Many researchers, after researching the drug resistance of TSCs, have found that the presence of TSCs in tumor tissues mainly leads to the failure of chemotherapy in cancer patients. TSCs, similar to adult stem cells, is endowed with the ability of self-renewal, which determines the tumorigenicity of TSCs. Therefore, carcinogenesis can be inhibited by prompting the differentiation of TSCs. TSCs play a key role in the metastasis, drug resistance and recurrence of malignant tumors. Hence, TSCs-targeted approaches may open up a promising and new path for anticancer treatment.

As one of the important methods for anticancer treatment, monoclonal antibody technology has been favored by many researchers in recent years. Sun Lichao, Zhao Xuan et al found that TSCs can be isolated from human orthotopic liver cancer tissues, and large-capacity functional monoclonal antibody libraries can be obtained drawing on spleen cell fusion technique. Monoclonal antibodies of four strains of anti-hepatoma TSCs were
obtained via tumorigenic experiments in animals. In vitro functional experiments revealed that these monoclonal antibodies can significantly inhibit the growth and proliferation of TSCs spheroid formation. In addition, in 2003, Tao Wei \(^5\) initially prepared monoclonal antibodies of anti-prostate stem cells by applying lymphocyte hybridoma technology. In 2013, Fan Hongyan \(^6\) and others observed from in vitro and in vivo functional experiments that the anti-prostate stem cell monoclonal antibody had a satisfactory inhibitory effect on prostate cancer. Further studies on stem cell monoclonal antibody of ovarian cancer \(^7\) and pancreatic cancer \(^8\) were carried out in succession, and meaningful research results have been achieved. Moreover, some scholars ploughed into a research from the aspect of TSCs micro-environment, and the treatment of TSCs-targeted local survival environments has gradually gained attention.

This study intends to draw on the research experience of predecessors and use the previously isolated HUSSLCs to immunize BALB/C mice for the purpose of establishing a functional monoclonal antibody library and screening out therefrom monoclonal antibodies with specific functions that recognize and inhibit HUSSLCs. And its nature was identified, and its function was studied.

**Methods**

**Source and preparation of cell lines**

The second generation CD133+ spheroid cells (named HUSSLCs) of SK-UT-1 cell line were from previous experiments and were stored in the cell center of Xiangya medical school. SP-2/0 myeloma cells were donated by Professor Liu Rushi of Hunan Normal University \(^9\)-\(^11\). Balb/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experiments were approved by the Experimental Animal Ethics Committee of the Medical College of Hunan Normal University.
**Determination of serum titer of HUSSLCs-immunized Balb/c mice**

Mouse immunization experiment and preparation of cell suspension

Two female 6-weeks old BALB/c mice were intraperitoneally injected with HUSSLCs 1×10^7/0.5 mL saline; the same immunization was performed every 2 weeks after each immunization, totaling three times. Two weeks after the third immunization, each mouse received intrasplenic injection of HUSSLCs 5×10^5/0.1 mL normal saline for impact immunization. After 3 days, spleen cell suspension was prepared.

**Detection of serum antibody titer by ELISA**

Two weeks after each immunization, orbital blood of mice was taken for detection of serum titer, and the serum was diluted with a phosphate buffer at a concentration gradient of 1:10, 1:100, 1:1000, 1:10000, 1:100,000, and 1:1000000 respectively for ELISA. The absorbance value was measured at a wavelength of 450 nm using enzyme-labelled spectrophotometer.

**Cell fusion and positive clone screening**

**Cell fusion**

One unimmunized BALB/c mouse was put to death and intraperitoneally injected with 5 ml of serum-free medium. The peritoneal fluid derived from softly rubbing the peritoneal cavity for 1 to 2 minutes was transferred to 20% FBS-HAT-1640 medium (400 ml) for culture.

One mouse immunized with HUSSLCs was selected and put to death. After that, its spleen was isolated and grinded for culture and collection of cell suspension, which was then treated to pellet-free particles.

SP-2/0 cells selected in logarithmic phase and the spleen cells after treatment were mixed and cultured. The cell mixture of 1000g was centrifuged for 2 minutes to remove the supernatant; 1ml of 37 °C PEG1500 was added to the precipitate and the cells were blown
and beaten for even mixture. 30ml serum-free medium was added after 1 minute, and the cell fusion was stopped; 1000g cell mixture after fusion was centrifuged for 2 minutes to remove the supernatant; the precipitate was resuspended using the above-prepared feeder cell-containing medium. The fused cells were inoculated into a 96-well culture plate at 200 μL/well, placed in an incubator, and the culture medium was changed once every 2 days. After 7 days of culture, HT containing (HT is the intermixture of hypoxanthine and thymidine) medium was used instead. When the cell colony covered 1/3-1/2 of the culture well, the next experimental operation was carried out.

**Clone screening**

50 μl/well of hybridoma cell culture supernatant was added to the ELISA plate. Two wells with no cell growth were chosen for each plate, which was set as a negative control, and another 2 wells with no cell growth added with positive serum was taken as a positive control; The serum of non-immune mice was taken as the negative control while in detection of antibody valence. The ELISA plate was incubated for half an hour at 37 °C. 100 ul of enzyme-labeled secondary antibody was added to each well and incubated at 37 ° C for half an hour. 200 ml of substrate was added to each well and developed at 37 °C for half an hour. The absorbance value (OD value) was measured at a wavelength of 450 nm using an enzyme-labelled spectrophotometer, which was compared with the OD value of the negative control well, with P/N greater than 2.1 serving as a critical point. After successful cell fusion and selective culture, the cell wells detected as positive via ELISA were prepared as cell suspension; the above cell suspensions were diluted at ratios of 1:2, 1:4, 1:8, 1:16, and 1:32 and inoculated into 96-well plates respectively. Each cell concentration was repeated through 8 wells to observe the growth of cells, with suspensions changed every two days; when the cell fusion degree reached 70%, the supernatant was taken for positive detection by ELISA; The positive single colony was
selected to repeat the process until the positive rate achieved 100% (clone culture 4 times); the positive clone cells were cultured in a common 24-well plate and a common 6-well plate respectively, which were eventually transferred to a 25 cm² culture flask for culture and amplification.

**Subtype identification of monoclonal antibodies**

A 96-well cell culture plate (flat bottom) was added with 50 μL 10 μg/mL of L-poly-L-Lysine per well, placed at room temperature for 30 minutes, and then washed twice with PBS. 50 μL of HUSSLCS cell suspension (2.5 x 10^6 cells/mL) was added to each well, placed overnight at 4 °C and washed once the next day with PBS. 50 μL of 0.5% glutaraldehyde was added to each well, fixed at 4 °C for 15 minutes, and washed twice with PBS. 400 μL of 0.1 mol/L glycine solution was added to each well, placed at 4 °C for 30 minutes, and washed 3 times with PBS. Incomplete DMEM medium was added each time and stored at -20 °C for later use. At the time of detection, the cell culture plate was taken out from the -20 °C refrigerator, and after the liquid in the well was melted, it was washed twice with PBS. 100 μL of the hybridoma cell supernatant to be tested was added to each well, placed at room temperature for 2 hours and washed 4 times with PBS. (1 hour incubation at 37 °C) 100 μL of enzyme-labeled secondary antibody (goat anti-mouse IgM, goat anti-mouse IgG, goat anti-mouse IgG1, goat anti-mouse IgG2a, goat anti-mouse IgG2b and goat anti-mouse IgG3) were added to each well at room temperature for 2 hours and washed 6 times with phosphate buffer (incubation at 37 °C for 30 min). 200 μL of freshly prepared substrate (TMB) was added to each well, placed at 37 °C for 30 minutes. The absorbance value was measured at a wavelength of 450 nm using an enzyme-labeled spectrophotometer.

**Identification of monoclonal antibodies**
HUSSLCs were taken and inoculated into a common 6-well plate; after cell fusion reached 70%, it was rinsed twice with PBS and fixed by 4% paraformaldehyde at room temperature for 10 minutes; rinsed 3 times for 3 minutes each time, and hybridoma cell culture supernatant was incubated at 37 °C for 1 hour; rinsed with PBS 3 times, 3 minutes each time, with goat anti-mouse-cy7-fluorescent secondary antibody (1:500 dilution) incubated at 37 °C for 1 hour; rinsed with PBS 3 times, 3 minutes each time, with DAPI (1:100, diluted with PBS) incubated and kept out of light at room temperature for 10 minutes, rinsed 3 times with PBS, 3 minutes each time; observed under microscope.

**Effect of monoclonal antibodies secreted by positive hybridoma cell lines on spheroid formation of HUSSLCs**

SP-2/0 cell supernatant and positive hybridoma cell supernatants with varied dilution ratios (1:1000, 1:500, 1:100) were used to act on HUSSLCs for 48 hours. The spheroid formation was performed as the method mentioned in the literature [9]. After 6 days of culture, non-adherent, clonal-growth tumor spheres were obtained, with a diameter ≥ 50.0 μm defined as tumor spheres. The number of tumor spheres in each well was counted. Spheroid formation rate = average number of tumor spheres per well / total number of viable cells inoculated × 100%.

**Effect of monoclonal antibodies secreted by positive hybridoma cell strains on the formation of HUSSLCs agar colonies**

SP-2/0 cell supernatant and positive hybridoma cell supernatants with varied dilution ratios (1:1000, 1:500, 1:100) were respectively chosen to act on HUSSLCs for 48 hours. DMEM medium with 20% FBS was proportionally mixed with 1.2% low melting point agarose fluid. The mixture was transferred to a 24-well plate, 0.5ml per well, and was used as bottom agar by the time the mixture was solidified. DMEM medium with 20% FBS was proportionally mixed with 0.6% low melting point agarose fluid. The mixture was
transferred to a 24-well plate, and 1000 HUSSLCs cells were added to each well for thorough mixture, which was then used as the top agar. After the top agarose fluid was solidified, it was transferred to an incubator for 14 days, and 500μl of DMEM medium with 20% FBS was added every 5 days. The number of cells ≥ 20 was defined as a colony, and the number of colonies was counted using a fluorescent inverted microscope. Colony formation rate = average number of colonies per well / total number of viable cells inoculated × 100%.

**Effect of monoclonal antibodies secreted by positive hybridoma cell strains on migration of uterine sarcoma stem cell-like cells**

SP-2/0 cell supernatant and positive hybridoma cell supernatants with varied dilution ratios (1:1000, 1:500, 1:100) were respectively chosen to act on HUSSLCs for 48 hours. The treated HUSSLCs cells were inoculated onto a 6-well cell culture plate and diluted to 5 × 10^5 cells/well with DMEM complete medium containing 10% fetal bovine serum, photographed when the cell fusion reached 90%. The tip head was used to scratch the bottom center of the 6-well plate. PBS was then adopted to rinse and remove the debris and floating cells for 2 times. The cells continued to be cultured after the scratches were made, photographed at the same wound site 24 hours later to count cells in the wound area. The cells treated with SP-2/0 cell supernatant were set as the control group, and the relative cell migration rate was calculated.

**Effect of monoclonal antibodies secreted by positive hybridoma cell strains on the expression of HUSSLCs CD133**

SP-2/0 cell supernatant and positive hybridoma cell supernatants with varied dilution ratios (1:1000, 1:500, 1:100) were respectively chosen to act on HUSSLCs for 48 hours, which was inoculated onto William's E medium (containing 20% FBS) at 10^5 cells/ml and
incubated for 15-30 minutes at room temperature to block non-specific sites. The cells were rinsed twice with PBS and resuspended in 990 μl of PBS. Then, 10 μl of antibody (including PE-CD133 and isotype control PE-IgG2b) was added to cell suspension. After incubating for 30 minutes at 4 °C away from the light, the cells were rinsed twice with PBS, fixed with 0.1% formaldehyde and detected by FACS Calibur™ system. All data was analyzed using Flow jo7.6.1 software.

**Effects of monoclonal antibodies secreted by positive hybridoma cell strains on the expression of HUSSLCs CD44, ABCG2, Bmi1, Nanog, Oct4 and ALDH1 proteins**

SP-2/0 cell supernatant and positive hybridoma cell supernatants with varied dilution ratios (1:1000, 1:500, 1:100) were respectively chosen to act on HUSSLCs for 48 hours. Whole-cell extracts were prepared. Bradford assay was applied to detect the protein content in cell lysates (supernatants). 40 μg of the extracted protein was taken for electrophoretic separation using SDS-polyacrylamide gel, and transferred to polyvinylidene difluoride membrane. The membrane was sealed with 5% bovine serum albumin for 2 hours at room temperature. The membranes were incubated overnight at 4 °C with respective antibodies as primary antibodies. The polyvinylidene difluoride membrane was washed with 1X Tris and incubated along with horse radish peroxidase secondary antibody for 2 hours at room temperature. Polyvinylidene difluoride membrane was washed with 1X TBS, and the protein expression was detected using enhanced chemiluminescence. The ratio between CD44, ABCG2, Bmi1, Nanog, Oct4, ALDH1 and β-actin protein band grayscales was respectively analyzed and calculated by image analysis software. The ratio between CD44, ABCG2, Bmi1, Nanog, Oct4, ALDH1 and β-actin protein band grayscales after HUSSLCs has been treated with SP-2/0 medium supernatant was defined as 1.00, which was standardized to relative density. The above experiment was repeated 3 times, and the data of 3 independent experiments were expressed as mean ±
standard deviation ($n = 3$).

**Statistical analysis**

Experimental data were expressed as mean ± standard deviation (Mean ± SD), with all performed using SPSS 18.0 software. LSD was adopted for comparison between homoscedasticity mean values, and Tukey's test for homoscedasticity mean values in multiple sets, with $P < 0.05$ suggesting statistical difference.

**Results**

**Experimental results of detection of HUSSLCs immunogenicity by ELISA**

Figure 1A showed that the antibody level in mice had lower valence after the first immunization, suggesting no significant difference from the negative control; after the second and third impact immunization, the antibody valence increased significantly ($P < 0.05$); after the third immunization, the anti-HUSSLCs antibody valence can reach 1.0 or more in a million-fold diluted immune serum. The myeloma SP2/0 cell line cells grew semi-adherently in complete 1640 medium of 10% fetal bovine serum (Fig. 1B). The cells were round, morphological integral and neatly arranged. After 3 days of culture, the degree of fusion could achieve 85%. After 8 days of screening through liquid culture medium, the fused hybridoma cells grew into clusters to form small cell colonies, with the cells mixed and translucent, proliferating in fast pace (see Fig. 1C). Positive hybridoma cells were detected by ELISA, and 6 positive wells were found. The culture well numbers were named 4-2, 9-15, 10-2, 10-4, 10-11 and 10-12, respectively. After 4 times cloning of positive wells by method of limiting dilution, it was found that 6 hybridoma cell strains could stably secrete anti-HUSSLCs monoclonal antibodies, named 1-2F, 1-2G, 1-3F, 1-9B, 1-3G, 3-3I respectively and frozen in conventional liquid nitrogen. As shown in Fig. 1D, 1-2F, 3-3I, 1-2G, 1-3G, 1-9B were IgG2a, and 1-3F was IgG2b. The results of cell immunofluorescence assay indicated that red fluorescence in varying degrees were displayed in HUSSLCs
incubated by monoclonal cell supernatants of 1-2F, 1-3F, 1-2G, 1-3G, 3-3I and 1-9B, among which 1-2F monoclonal cell supernatants incubated the highest number of positive cells labeled red fluorescence, followed by 1-3G monoclonal cell supernatants, reaching more than 50% (Fig. 1E).

**Effect of monoclonal antibodies on HUSSLCs spheroid formation, agar colony and HUSSLCs migration**

The results showed that supernatants of 1-2F monoclonal cells with different dilution ratios (1:1000, 1:500, 1:100) reduced HUSSLCs spheroid formation rate (Fig. 2A, B), agar colony formation rate (Fig. 2C, D), cell migration rate (2E, F) in concentration-dependent manner, with all P < 0.05.

**Effect of monoclonal antibodies on CD133 expression of HUSSLCs**

1-2F monoclonal cells supernatants with varying concentrations (1:1000, 1:500, 1:100) reduced CD133 positive cells expression of HUSSLCs in concentration-dependent manner (Fig. 3A, B, P < 0.05). Results showed that all three concentrations showed a significant reduction effect on CD133 positive cells expression.

**Effects of monoclonal antibodies on the expression of CD44, ABCG2, Bmi1, Nanog, Oct4 and ALDH1 proteins**

Western blot analysis showed that 1-2F monoclonal cell supernatants (1:1000, 1:500, 1:100) with varying dilution ratios reduced expression levels of HUSSLCs CD44, ABCG2, Bmi1, Nanog, Oct4, ALDH1 protein in concentration-dependent manner. (Fig. 3C, P < 0.05). For ABCG2 and ALDH1, all three concentrations showed a significant reduction effect on protein expression. But for CD44, Bmi1, Nanog and Oct4, only concentrations of 1:500 and 1:100 showed a significant reduction effect, while the concentration of 1:1000 showed no significant difference.

**Discussion**
Since the successful isolation of Tumor Stem Cells (TSCs) from human acute myeloid leukemia in 1997 by Bonnet et al.\(^\text{12}\), TSCs were isolated from a variety of malignant tumors such as breast cancer, pancreatic cancer, prostate cancer, and liver cancer. Through a series of studies, it is found that TSCs are closely related to drug resistance, metastasis and recurrence of malignant tumors. Therefore, TSCs targeted treatment is expected to become a new and effective approach for malignant tumors.

The spheroid formation rate was determined by referring to the methods and procedures of literature \(^\text{4, 9-11, 13}\). The results revealed that the supernatants of 1-2F monoclonal cells with different dilution ratios reduced the rate of HUSSLCs spheroid formation in concentration-dependent manner, indicating that 1-2F monoclonal antibody has the effect of inhibiting self-renewal of HUSSLCs. Agar colony formation is commonly used to detect anchorage-independent growth and anoikis tolerance of tumor stem cells\(^\text{14}\) and is positively correlated with the potential in vivo tumor formation of tumor cells\(^\text{15}\). The results of agar colony formation rate assay showed that the supernatants of 1-2F monoclonal cells with different dilution ratios reduced the rate of HUSSLCs agar colony formation in concentration-dependent manner, indicating that 1-2F monoclonal antibodies can inhibit anchorage-independent growth of HUSSLCs and has tumorigenic effect. Studies have proved that tumor stem cells exhibit an Epithelial-Mesenchymal Transition (EMT) phenotype with enhanced in vitro cell migration, invasion and metastasis in vivo\(^\text{13, 16-21}\).

The assay results of cell migration rate using wound healing method demonstrated that supernatants of 1-2F monoclonal cells with different dilution ratios reduced HUSSLCs cell migration rate in concentration-dependent manner, suggesting that 1-2F monoclonal antibodies have the effect of inhibiting the migration and motility of HUSSLCs. CD133, a transmembrane glycoprotein, is an important surface marker of stem cells and tumor stem
cells of various tissues including uterine sarcoma and is associated with poor prognosis in patients with various types of tumors. Analysis results of PE-labeled CD133 antibody flow cytometry revealed that the supernatant of 1-2F monoclonal cells with different dilution ratios reduced the percentage of CD133-positive cells of HUSSLCs in concentration-dependent manner, indicating that the supernatant of 1-2F monoclonal cell culture medium can inhibit the expression of CD133 protein of HUSSLCs tumor stem cell marker or can effectively reduce the number of tumor stem cells. Ample studies have shown that CD44 overexpression can facilitate and maintain TSC self-renewal, migration and invasion, serving as a marker protein for a variety of TSC; ABCG2 overexpression contributes to and maintains multidrug resistance of tumor stem cells; Abnormal expression of Bmi1 contributes to and maintains the self-renewal, unrestricted proliferation and multidrug resistance properties of tumor stem cells; Nanog abnormal expression and functional effect of self-renewal and unrestricted proliferation of tumor stem cells; Oct4 abnormal expression is associated with self-renewal and unrestricted proliferative function of tumor stem cells, as well as epithelial-mesenchymal transition phenotype; ALDH1 abnormal expression enhances the cytotoxic effects of tolerant drugs of stem cells and tumor stem cells, as well as other compounds, contributing to and maintaining multidrug resistance of tumor stem cells and cell anoikis resistance. Western blot analysis showed that the supernatants of 1-2F monoclonal cells with different dilution ratios reduced the expression levels of CD44, ABCG2, Bmi1, Nanog, Oct4 and ALDH1 proteins of HUSSLCs in concentration-dependent manner. These results indicate that 1-2F monoclonal antibodies can inhibit the expression of the above various tumor stem cell markers of HUSSLCs, or can effectively inhibit the self-renewal, unrestricted proliferation, migration, invasion, and multidrug
resistance of HUSSLCs and other tumor stem cell characteristics.

So far there are no reports concerning the functional monoclonal antibodies of uterine sarcoma stem cells at home and abroad. Our research group is currently conducting the next in vivo functional test, using cell tumorigenic nude mice containing varied tumor markers, and then adopting positive monoclonal antibodies to perform antibody treatment experiments on tumorigenic nude mice. The study intends to conduct in-depth exploration of whether these functional monoclonal antibodies can effectively inhibit the growth and metastasis of uterine sarcoma xenografts and prolong the survival of tumor-bearing mice in an attempt to provide alternative therapeutic agents with application values for HUSSLCs-targeted treatment, and to lay a foundation for the identification of new molecular targets of HUSSLCs.

Anti-HUSSLCs monoclonal antibody features functions of inhibiting the self-renewal, unrestricted proliferation, migration, invasion, and multidrug resistance of HUSSLCs as well as other characteristics of tumor stem cells.

Conclusion

In conclusion, our study demonstrates that antibody valence produced by HUSSLCs-immunized mice meet the requirements for the preparation of monoclonal antibodies. Anti-Husslcs monoclonal antibody has the function of inhibiting self-renewal of HUSSLCs, unrestricted proliferation, migration, invasion and multi-drug resistance, as well as the function of tumor stem cells. It provides hope and possibility for targeted therapy of HUSSLCs.

Declarations

**Ethics approval and consent to participate**

All animal experiments were approved by the Experimental Animal Ethics Committee of
the Medical College of Hunan Normal University.

Consent for publication

Not applicable.

Availability of data and material

No additional data are available.

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Jiuping Gao and Beilei Zhang carried out the studies, participated in collecting data, and drafted the manuscript so they should be regarded as joint first authors. Dihong Tang, Ting Gao, Qiuhui Lin, Jun Tang, Chaonan Chu and Ting Yang performed the statistical analysis and participated in its design. Jing Wang, Jingting Cai and Jing Wang helped to draft the manuscript. All authors read and approved the final manuscript.

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Abbreviations

Human Uterine Sarcoma Stem Cell-Like Cells: (HUSSLCs); Tumor Stem Cells: (TSCs);
Epithelial-Mesenchymal Transition: (EMT).

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Figures
Figure 1

A Detection of serum antibody titer of HUSSLCs-immunized Balb/c mice by ELISA

B Semi-adherent growth image of SP-2/0 cells under inverted phase contrast microscope (×20) C Single-layer adherent growth image of hybridoma cells fused under inverted phase contrast microscope (×20) D Detection of specific antibody-secreting subtypes of positive hybridoma cells by ELISA E Detection of cellular immunofluorescence images of HUSSLCs by monoclonal antibodies (×20)
A, Monoclonal cells reduced HUSSLCs spheroid formation ability (×100). B, Spheroid formation rate detection. Compared with SP-2/0 Cell supernatant treated cells (Cont): * P <0.05 (mean±SD, n=3); Compared with 1-2F Monoclonal cell supernatant (1:1000) treated cells: # P <0.05 (mean±SD, n=3). C, Monoclonal cells reduced HUSSLCs agar colony formation rate (×100). D, agar colony formation rate detection. Compared with SP-2/0 cell supernatant treated cells (Cont): * P <0.05 (mean±SD, n=3); Compared with 1-2F monoclonal cell supernatant (1:1000) treated cells: # P <0.05 (mean±SD, n=3). E, Monoclonal cells reduced HUSSLCs cell migration rate (×100). F, Cell migration rate detection. Compared with SP-2/0 cell supernatant treated cells (Cont): * P <0.05 (mean±SD, n=3); Compared with 1-2F monoclonal cell supernatant (1:1000) treated cells: # P <0.05 (mean±SD, n=3).
A, Flow cytometry analysis image indicated that monoclonal cells reduced HUSSLc CD133 positive cells. B, CD133 positive cell percentage determination. Compared with SP-2/0 cell supernatant treated cells (Cont): * P <0.05 (mean±SD, n=3); Compared with 1-2F monoclonal cell supernatant (1:1000) treated cells: # P <0.05 (mean±SD, n=3). C Western blot results of expression of CD44, ABCG2, Bmi1, Nanog, Oct4 and ALDH1 proteins. Note: Compared with SP-2/0 cell supernatant treated cells (Cont): * P <0.05 (mean±SD, n=3); Compared with 1-2F monoclonal cell supernatant (1:1000) treated cells: # P <0.05 (mean±SD, n=3)