Characterization of cancer stem-like cells in the side population cells of human gastric cancer cell line MKN-45*

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Abstract: Objective: Side population (SP) cells may play a crucial role in tumorigenesis and the recurrence of cancer. Many kinds of cell lines and tissues have demonstrated the presence of SP cells, including several gastric cancer cell lines. This study is aimed to identify the cancer stem-like cells in the SP of gastric cancer cell line MKN-45. Methods: We used fluorescence activated cell sorting (FACS) to sort SP cells in the human gastric carcinoma cell line MKN-45 (cells labeled with Hoechst 33342) and then characterized the cancer stem-like properties of SP cells. Results: This study found that the SP cells had higher clone formation efficiency than major population (MP) cells. Five stemness-related gene expression profiles, including OCT-4, SOX-2, NANOGL, CD44, and adenosine triphosphate (ATP)-binding cassette transporters gene ABCG2, were tested in SP and MP cells using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). Western blot was used to show the difference of protein expression between SP and MP cells. Both results show that there was significantly higher protein expression in SP cells than in MP cells. When inoculated into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, SP cells show higher tumorigenesis tendency than MP cells. Conclusions: These results indicate that SP cells possess cancer stem cell properties and prove that SP cells from MKN-45 are gastric cancer stem-like cells.

Key words: ATP-binding cassette transporters, Side population cells, Stem cells, Benzimidazole (Hoechst 33342), Stomach neoplasm


1 Introduction

Cancer is one of the major causes of mortality after acute contagious diseases, cardiovascular and cerebrovascular diseases. The higher mortality rate in cancer is due to increased cases of relapse and metastasis. Cancer stem cell (CSC) hypothesis has received more and more attention in recent times for its better explanation of the initiation of relapse and metastasis in several types of carcinomas including gastric carcinoma (Reya et al., 2001; Houghton et al., 2004; McDonald et al., 2008). In recent research, several malignant tumor tissues and cell lines have been discovered to possess CSCs, and include acute lymphoblastic leukemia and several solid tumors like breast, colon, hepatic, prostate, lung, and gastric cancers (Alvi et al., 2003; Chiba et al., 2006; Ho et al., 2007; Ricci-Vitiani et al., 2007; Fukuda et al., 2009; Oates et al., 2009). This has strengthened the
hypothesis that the initiation of relapse and metastasis may be caused by CSCs.

Gastric cancer is still the 4th most common cancer and the 2nd leading cause of cancer mortality all over the world. There are about one million new cases per year worldwide and 850,000 deaths from the disease. The incidence in most western countries lies between 10 and 15 new cases per 100,000 people per year. However, Japan, Korea, and China now lead with up to 80 new cases per 100,000 people per year (Parkin et al., 2005; Krejs, 2010). The most effective and specific methods available to deal with the disease include local resection, chemotherapy, and radiotherapy. The identification and isolation of the CSCs, which possess higher specificity to a particular type of cancer, is being looked to as an important step to improve the therapeutic options in the treatment of cancer. Recently, some researchers used fluorescence activated cell sorting (FACS) to differentiate side population (SP) and major population (MP) cells, mainly by employing the nucleic acid dye Hoechst 33342 to stain the cancer cells (Goodell et al., 1996; Huang et al., 2009). Similar methods may be applied in some tumors whose stem cell markers are not known. Our study used this method to sort SP cells from gastric cancer cell lines and then tested the molecular characteristics and biological behavior of these cells. Our study established that SP cells were gastric cancer stem-like cells.

2 Materials and methods

2.1 Cell culture

Human gastric adenocarcinoma cell line MKN-45 was obtained from the Cancer Institute, Chinese Academy of Medical Sciences and was separately maintained in Royal Park Memorial Institute (RPMI) 1640 (Invitrogen, USA) supplemented with 10% (0.1 g/ml) fetal bovine serum (FBS), 100 U/ml penicillin G, and 100 µg/ml streptomycin. Both kinds of cells were maintained at 37 °C in a humidified 5% CO2 incubator.

2.2 Fluorescence activated cell sorting

Near confluent cells from MKN-45 were harvested by trypsinization with 0.25% (2.5 g/L) trypsin ethylenediaminetetraacetic acid (EDTA; Invitrogen, USA), centrifuged at the rate of 1000 r/min for 10 min, washed for two times with phosphate buffered saline (PBS), resuspended at 1×10^6 cells/ml in pre-warmed 37 °C medium of RPMI 1640 with 2% (0.02 g/ml) fetal calf serum (FCS), and passed through 40 µm cell strainers (BD Falcon, USA) to get single-cell suspensions. The cells were then labeled with Hoechst 33342 (Sigma-Aldrich, USA) at a concentration of 5 µg/ml. The labeled cells were incubated in the dark for 60–75 min in a 37 °C water bath with intermittent mixing, either alone or with 75 µmol/L verapamil (Sigma-Aldrich, USA). The cells were suspended on ice in PBS containing 2% FBS after staining and maintained at 4 °C until flow cytometry analysis. Cells were labeled with 1 µg/ml propidium iodide (PI) to assess viability 5 min before examination. The stained cells were analyzed using a FACS Aria II (BD Biosciences, San Jose, CA, USA). The Hoechst dye was excited by ultra-violet laser at 375 nm and its fluorescence measured with 450/40 nm (Hoechst blue) and 695/40 LP (long-pass, Hoechst red) optical filters.

2.3 Cell growth curve and clone formation assays

Fresh sorted SP and MP cells of MKN-45 were incubated immediately at 2×10^5 cells per well in 96-well plates, at a total volume of 200 µl. Each subpopulation had 10 replicates cultured in RPMI 1640 with 10% (0.1 g/ml) FBS. The culture medium was removed each day during the following 7 d and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was performed in a routine manner. The procedure was repeated three times. We plotted the growth curve according to the absorbance of each well from a Bio-Rad enzyme reader at the wavelength of 570 nm.

SP and MP cells were counted and plated in 6-well plates at 250 cells per well in triplicate after sorting, and then cultured in RPMI 1640 with 10% FBS for 10–14 d. When most cell clones reached more than 50 cells, they were washed twice with PBS, fixed in methanol for 15 min, and stained with crystal violet dye for 15 min at room temperature. The number of colonies containing more than 50 cells was counted. The colony formation efficiency (CFE) was calculated via colony number/seeding cell number×100%, and the results compared.