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Ovarian cancer cells with the CD117 phenotype are highly tumorigenic and are related to chemotherapy outcome

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ABSTRACT

Cancer stem cells (CSCs) play an important role in the recurrence and drug resistance of cancer. Isolation and characterization of CSCs from ovarian cancer samples may help to provide novel diagnostic and therapeutic targets in the management of recurrent disease and drug resistance in ovarian cancer. Here, we developed a xenograft model in which cells from 14 samples of human ovarian serous adenocarcinoma tissue or ascites were implanted in immunodeficient mice to test the tumorigenic potential of different populations of ovarian cancer cells. We identified and isolated the tumorigenic cells as $CD117^+Lineage^-$ from three different xenografts. As few as 10^3 cells with the $CD117^+Lineage^-$ phenotype, which comprise <2% of the xenograft tumor cells, were able to regenerate tumors in a mouse model, a 100-fold increase in tumorigenic potential compared to $CD117^-Lineage^-$ cells. The tumors that arose from purified $CD117^+Lineage^-$ cells reproduced the original tumor heterogeneity and could be serially generated, demonstrating the ability to self-renew and to differentiate, two defining properties of stem cells. Furthermore, immunohistochemistry analysis of 25 patients with advanced ovarian serous adenocarcinoma revealed positive immunostaining for CD117 in 40% (10 of 25) of patients. CD117 expression was statistically correlated with resistance to conventional chemotherapy ($P = 0.027$). In conclusion, our study demonstrates that human ovarian cancer cells with the $CD117^+$ phenotype possess the unique properties of CSCs, including self-renewal, differentiation, a high tumorigenic potential, and chemoresistance. Future studies designed to target $CD117^+$ cancer cells may identify more attractive and effective therapies for treatment of ovarian cancer.

Introduction

Ovarian cancer is the fifth leading cause of cancer death and the most lethal malignancy of the female reproductive system. Although the standard therapy of cytoreductive surgery followed by platinum-paclitaxel chemotherapy results in complete remission in 70% of patients, most will relapse within 2 years due to chemoresistance (Ozols, 2005).

The cancer stem cell (CSC) theory suggests that only a subset of cancer cells (CSCs) possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor (Bonnet and Dick, 1997; Clarke et al., 2006; Reya et al., 2001). In addition, CSCs have been shown to be a population of drug resistant cells that can survive chemotherapy in various types of tumor (Al-Hajj et al., 2004; Dean et al., 2005; Jordan et al., 2006; Todaro et al., 2007). This model might explain why standard chemotherapy may shrink tumors, but most tumors regrow and eventually cause a relapse. Thus, the specific identification and targeting of CSCs could be highly valuable for eradicating cancer and resolving problems of drug resistance and recurrence.

Pioneering work in the identification and isolation of CSCs was undertaken in acute myeloid leukemia (Bonnet and Dick, 1997; Lapidot et al., 1994) and, later, in various solid tumors including breast cancer (Al-Hajj et al., 2003), brain cancer (Singh et al., 2004), colon cancer (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007), head and neck squamous cell carcinoma (Prince et al., 2007), pancreatic cancer (Li et al., 2007), lung cancer(Eramo et al., 2008), liver cancer (Yang et al., 2008), and melanoma (Schatton et al., 2008). There were also a few studies on ovarian CSCs. Moserle et al. identified a sub-population of ovarian cancer cells with a side population (SP) phenotype from one xenograft tumor. These cells were highly tumorigenic in mice compared to non-SP cells (Moserle et al., 2008). Zhang et al. observed that a sub-population of primary human ovarian cancer cells could form anchorage-independent, self-renewing spheres *in vitro* with a high expression of CD44 and CD117. Cells with the $CD44^+CD117^+$ phenotype were highly tumorigenic and capable of re-establishing their original tumor hierarchy when injected into mice (Zhang et al., 2008). Baba et al. reported that $CD133^+$ cells from

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ovarian cancer cell lines exhibited enhanced resistance to platinum-based therapy and formed more aggressive tumors in mice compared to $CD133^-$ cells (Baba et al., 2009). In another study, Gao et al. also identified a sub-population enriched for ovarian CSCs and defined by the CD24 phenotype in a series of cancer cell clones (Gao et al., 2010). Cell surface markers CD44 (Al-Hajj et al., 2003; Dalerba et al., 2007; Li et al., 2007; Prince et al., 2007; Zhang et al., 2008), CD24 (Gao et al., 2010; Li et al., 2007), and CD133 (Baba et al., 2009; O'Brien et al., 2007; Ricci-Vitiani et al., 2007; Singh et al., 2004) were confirmed as CSC markers respectively in a variety of tumor types, while single surface marker CD117 has seldom been reported.

CD117(C-kit) is a surface marker for embryonic stem cells, hematopoietic stem cells, and mesenchymal stem cells, it maintains cells in their undifferentiated state, and it confers the ability to self-renew (Hassan and El-Sheemy, 2004; Lu et al., 2007; Palmqvist et al., 2005). CD117 was also observed in tumors of both mesenchymal origin and epithelial origin. Cancer patients with over-expression and/or mutations of CD117 have significantly poorer prognoses in numerous clinical studies of various tumor types (Cohen et al., 1994; Curtin et al., 2006; Duensing et al., 2004; Hoei-Hansen et al., 2007; Naeem et al., 2002; Tsuda et al., 2005; Yasuda et al., 2006). However, how CD117 is involved in ovarian cancer is unclear. Recently, the CSC hypothesis may shed light on its role in the development and growth of ovarian cancer.

In the current study, using methods successfully used previously to identify CSCs in breast cancers (Al-Hajj et al., 2003), we directly injected cells isolated from human ovarian serous carcinoma samples into immunodeficient mice and eliminated non-tumor cells and cultured tumor cells in vivo to assess the characteristics of the CD117 phenotype in detail. The $CD117^+$ ovarian cancer cells showed the exclusive ability to consistently form tumors in mice and recreate the original tumor heterogeneity. Moreover, immunohistochemistry (IHC) analysis of clinical paraffin-embedded specimens showed that $CD117^+$ patients were less sensitive to first-line chemotherapy and recurrence within a shorter period compared to $CD117^-$ patients. Our findings may give the great contribution of CD117 to the pathogenesis of ovarian cancer, which may help develop valuable therapeutic approaches to treat ovarian cancer.

Materials and methods

Collection of ascites and fresh tumor specimens

Fresh ascites (500 ml) and tumor tissues from the ovary and the omentum were obtained within 30 min following abdominocentesis or surgical resection from 14 newly diagnosed patients with advanced ovarian serous adenocarcinoma at the Department of Gynecology, Peking Union Medical College Hospital (PUMCH) between December 2008 and November 2009. This study was approved by the Ethics Committee of PUMCH, and full informed consent was obtained from all patients before the surgery.

Preparation of nude mice

Female BALB/c-nu mice that were 4–5 weeks old were purchased from the Institute of Laboratory Animal Sciences of the Chinese Academy of Medical Science & Peking Union Medical College (CAMS & PUMC), which is certified by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, USA). The mice were maintained in micro-isolator cages at the Institute. Animal study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of PUMCH.

Fresh specimen digestion and implantation

Fresh tumors were minced completely using sterile scalpel blades, suspended in DMEM/F12 medium (M&C GENE TECH, Beijing, China), and mixed with 1 mg/ml of collagenase IV (Sigma-Aldrich, St. Louis, MO) and 0.04% DNase I (Solabio, Beijing, China) followed by a 1 h incubation in a shaking bath with a constant temperature oscillator (Honghua Instrument Factory, Jintan, Jiangsu, China). Enzymatically disaggregated suspensions were filtered through a 100 μm cell strainer and washed twice with PBS; dead cells, debris and red blood cells (RBCs) were removed using Lymphocyte Separation Medium (Tbdscience, Tianjin, China). Ascites were collected and washed with PBS using the low-speed centrifugation method until the majority of RBCs were removed. Tumor cells in 200 μl of media were injected intraperitoneally (i.p.) into the nude mice, or cells in 100 μl media with Matrigel (BD Biosciences, Franklin Lakes, NJ) (1:1) were injected subcutaneously (s.c.) into the nude mice. Tumor growth was monitored once a week. The mice were euthanized when the tumors had grown to a maximum of 2000 mm^3 (s.c.) or when large ascites appeared (i.p.).

Preparation of single cell suspensions of tumor cells

Xenograft tumors were harvested, cut up into small pieces with scissors and then minced completely using sterile scalpel blades. To obtain single cell suspensions, tumor pieces were digested with 1 mg/ml collagenase IV and 0.04% DNase I for 1 h. Enzymatically disaggregated suspensions were filtered through a 100 μm cell strainer and washed with PBS as described above. Ascites were collected from mice and washed with PBS by the low-speed centrifugation method until the majority of the RBCs were removed. When a large number of agglomerated cells were present, 0.05% trypsin and 0.02% EDTA were used to digest the agglomeration into single cells at room temperature for 3 min (0.04% DNase I was added to avoid floccule formation). After cell digestion was complete, cells were filtered through a 40 μm nylon mesh and washed with PBS. Dead cells, debris and RBCs were removed using the Lymphocyte Separation Medium.

Flow cytometry

Cells were counted and then transferred to a 5 ml tube, washed twice with PBS containing 2% bovine serum albumin (BSA), and resuspended in 100 μl (per 10^6 cells) of PBS/2% BSA. Then, 5 μl of Human TruStain FcX™ (Fc Receptor Blocking Solution, Biolegend, San Diego, CA) was added, and samples were incubated at room temperature for 10 min, after which the cells were washed with PBS/2% BSA and re-suspended in 100 μl (per 10^6 cells) of PBS/2% BSA. Appropriately diluted antibodies were then added and samples were incubated for 10–30 min on ice according to instructions, followed by washing twice with PBS/2% BSA. When needed, a secondary antibody (streptavidin-FITC, eBioscience, San Diego, CA) was added. Cells were analyzed and sorted by flow cytometry (Moflo Beckman Coulter, Fullerton, CA, USA). Forward scatter area versus pulse width profiles was used to eliminate cell doublets and debris. Antibodies used in this study included: anti-human CD117-PE-CY5 (BD Pharmingen, Franklin Lakes, NJ), mouse IgG1 κ Isotype Control-PE-CY5 (BD Pharmingen), and anti-mouse $H\text{-}2K^d$-FITC (BD Pharmingen). Lineage (Lin) markers including biotin labeled anti-human CD2, CD3, CD10, CD16, CD31, CD64 (all were purchased from eBioscience), and CD140b (AbD Serotec, Oxford, UK) were used for the identification of normal human leukocytes, endothelial cells, mesothelial cells, and fibroblasts (Al-Hajj et al., 2003). In all experiments, human non-tumor cells and mouse cells were removed by eliminating Lin^+ and $H\text{-}2K^{d+}$ cells.

Implantation of unsorted and sorted cells into nude mice

Different numbers of unsorted or sorted $CD117^+Lin^-$ or $CD117^-Lin^-$ cells were suspended in media/Matrigel mix (1:1) and injected s.c. into female BALB/c-nu mice or i.p. into the abdominal cavity of the mice. Tumor growth was monitored twice a week for