The cancer stem cells (CSCs) are regarded as a critical explanation for tumor initiation, metastasis and therapeutic failure. CSCs possess similar biological traits of normal stem cells such as self-renewal, differentiation and trans-differentiation. Esophagus adenocarcinoma (EAC) and esophagus squamous cell carcinoma (ESCC) are two histological types of esophageal cancers. In order to investigate the roles of CSCs in ESCC, the variant cells were examined by Ultra-Low Attachment culture system. The cells were seeded in plate, which was hydrophilic, neutrally charged coating covalently bound to a polystyrene vessel surface with DMEM/F12 supplemented with N2 supplement, FGF and EGF to form tumor sphere. About 12% of tumor spheres were generated with 100 to 300 mm for seven days. Due to CSCs accompany with upregulation of stemness and drug-resistance related genes, these genes were used to evaluated the features of CSCs. The data showed that most of stemness-associated genes (such as Oct4, Sox2 and Nanog) and drug-resistance related genes (such as ABCB1 and ABCG2) in tumor spheres with high levels compared with the parental cells. Notably, the series passage could enrich the population of CSCs, suggesting that the tumor spheres harbored self-renewal property in ESCC cells. CSCs hypothesis suggests that CSCs were able to differentiate into cancer cells and contributes to the heterogeneity of tumors. To examine the differentiation ability of tumor spheres, the tumor spheres of ESCC cells were reseeded in polystyrene plates with DMEM medium supplemented with 10% FBS to induce cell differentiation. The spheroid cells were re-attached into plate during the culture period. The stemness-associated and drug-resistance were significantly decreased after tumor spheres differentiation. In addition, the spheroid cells could transdifferentiate to endothelial cells that was confirmed the CSCs feature in spheroid cells. CSCs also accompany with tumor-initiating and metastasis capability, our data showed that even less than 10 spheroid cells could rise tumors in 2 of 4 mice and metastasized to distal organ compared to parental cells. In summary, the CSCs might cause tumor-initiating, metastatic ability and therapeutic failure in ESCC. Understanding the features and detail underlying mechanisms of CSCs might help for developing novel therapeutic strategy for ESCC patients.