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学位論文題目	CD24+SSEA4+ ovarian carcinoma cells exhibit self -renewal ability and tumorigenicity **（CD24+SSEA4+ヒト卵巣癌細胞は癌幹細胞の性質を持つ）**

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論　文　内　容　の　要　旨

Introduction

Ovarian cancers are a major cause of mortality worldwide. Because almost of them have becomes resistant to chemotherapy after surgical debulking. More recently, it has been hypothesized that functional heterogeneity in the tumor may account for the fact that not all of the cancer cells in solid tumors have a similar ability to drive tumor formation (Reya T et al., 2001). It has led to the cancer stem cell (CSC) hypothesis. CSCs are defined as a minority cell type in the tumor, which retains the capacity, through asymmetric division, for self-renewal as well as differentiation into multiple cell types. Through this process, CSCs can regenerate the entire tumor phenotype and subsequent metastases. Relatively successful cancer treatments shrink the bulk of tumor cells, but of the fail to eliminate the CSCs (Cabanilas R et al., 2009).

Attention has been paid to CSCs as a new target cell for treating cancer. However, no method of identifying specific CSCs has been established in primary human ovarian carcinoma. The aim of this research was to identify a new combination of surface markers for human ovarian CSCs.

Materials and Methods

The tumor tissues were obtained from different ovarian cancer patiens during surgical procedures. The study was approved with policites of the Research Ethics Committee of the University of Toyama and consent was obtained from the patient. After the cells isolated, they have been keeping in the culture and transplanted serially into subcautaneous of mice. The isolated cells were classified according to the cell surface marker, CD24 and/or SSEA4 with MACS (separator Magnetic cell sorting). They were stained with immunocytochemistry and analyzed with PCR for stemness marker. Also it was examined for sphere formation, flow cytometric (FCM) analysis, colony assay, chemotherapy resistance assay and tumorigenicity with xenotransplantation.

Results

By using RT-PCR methods, we found that the cultured cells strongly expressed stemness genes, such as *c-myc, oct3/4, sox2, nanog, abcg-2* and *bmi-1*. They also expressed surface markers such as CD24, SSEA4, CD133 and CD47. However, there are no big difference about the rate of gene expression between CD133+ and CD133-. The sub-population of CD24+ SSEA4+ cells expressed Oct-4 strongly, and was able to form spheres much more than CD24 single positive cells or CD24 and SSEA4 double negative cells. Only five CD24+SSEA4+ cells made spheres within three weeks. This sub-population formed the most numbers of colonies in the soft agar colonies assay. The CD24+SSEA4+ cells grew better in the presence of cisplatin better than other sub-populations. With injection of only

100 cells per mouse, CD24+SSEA4+ cells were tumorigenic in all scid mice (4/4), with two months.

Discussion

We described the isolation and characterization of a highly tumorigenic subpopulation of cells, cancer stem cells (CSCs), from human ovarian adenocarcinomas. Five separate criteria have been established for CSCs: a) self-renewal, b) small minority of the total tumor population, c) reproducible tumor phenotype, d) multipotent differentiation into nontumorigenic cells, and e) distinct cell surface antigenic phenotype, permitting consistent isolation (Dalerba P et al., 2007. Clarke MF et al., 2006).

In the present study, we selected subpopulation from primary ovarian cancer as CD24 and SSEA4 for the marker of CSCs. CD24 has been used as a cell surface marker of CSCs in hepatocarcinoma, prostate, clearcell renal carcinoma and ovarian cancer. SSEA4 is known as the stemness marker on the ES or iPS cells, but there are no reports about CSCs. The expression of SSEA-4 was found to increase from normal epithelium to benign cystademona and to borderline cystadenoma and adnocarcinoma in both serous mucinous groups. Loss or reduction of the expression of SSEA-4 was significantly correlated with more advanced tumor stage and poorer tumor cell differentiation. This result was the opposite of our data. Nato et al. (2013) reported that CD44 and SSEA4 positive cells in an oral cancer cell live HSC-4 possess cancer stem cell characteristics. And SSEA4 was considered to be a new marker for cancer stem cell. CD133 is considered to be a cell surface marker of CSCs in melanoma, ovarian cancer and so on, too. However, our data was not difference the rate of stemness related gene expression between CD133+ and CD133-.

CD24+SSEA4+ cells were tumorigenic in all scid mice (4/4), with 2 month. In contrast no tumor was observed when 5.0×10^5 cells of CD24- cells. They were 5,000 times more tumorigenic than CD24- cells. In accord with the hypothesis that CSCs are resistant to chemotherapy and radiotherapy these standard therapies fail to completely eradicate CSCs resulting in recurrence. We showed that the CD24+SSEA4+ cells were more resistant to cisplatin than other types. These results suggested that these CD24+SSEA4+ cells in ovarian cancer could survive under the exposure to the existing anti-cancer therapies and cause recurrence.

Conclution

Based on these findings, it is suggested that CD24 and SSEA4 double positive cells have the characteristics of human ovarian tumor CSCs. It should be possible to develop an effective new clinical therapy using these cells as a target cell for human ovarian cancer.