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# Expression of CD133, CD44, CK7, and OCT4 in Animal Cancers

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**Abstract :** Cancers are mainly sustained by a small pool of neoplastic cells, known as cancer stem cells or tumor-initiating cells. These cells possess the ability to self-renew and proliferate, and are thus able to form the tumor. In the present study cells that correspond to cancer stem cells in mammary and liver cancers in animals were identified by the expression of CD133, CD44, CK7, and OCT4 using immunochemistry. As a result, we found with CD133+ and CD44+ cancer stem cell-like phenotypes in mouse and canine hepatocellular carcinoma and canine mammary gland tumors. However, CK7+ and OCT4+ cells were not identified in animal mammary and liver cancer. CD133+ and CD44+ cells are wellknown stem cell lines and play key roles in development and metastasis in human cancer. These findings suggest that cancer stem cells are involved in animal tumorigenesis and may provide insight into mechanisms in cancer development as well as cancer diagnostics.

**Keywords :** cancer stem cells, CD133, CD44, CK7, OCT4

## Introduction

Stem cells are characterized by the ability to self-renew through mitotic cell division and differentiate into a diverse range of specialized cells with characteristics similar to cells of various tissues [26]. During embryonic development, stem cells are very active and it presents in a bone marrow the most [11]. Adult stem cells are able to self-renew and differentiate into one or more specialized cell types [10]. In normal tissue, stem cells maintain organ and tissue regeneration [24].

Until recently, it was believed that the each cell within a tumor is tumorigenic. However, recent studies revealed that the all tumor cells do not have the ability to initiate cancer [29]. Some cells in tumors which have stem cell properties and can form tumors have been identified and are called cancer stem cells. They are able to initiate tumor growth and play a key role in tumor development and metastasis [7].

The features shared by both normal and cancer stem cells include ability to asymmetrically divide, self-renewal regulation by similar signaling pathways, arrangement progenitor and differentiating cells, longer telomeres and telomerase activity which lengthens the life-span of the cell, and expression of the ATP-binding cassette transporter (ABC-transporter) that makes cells resistant to growth-inhibition drugs [11].

Identifying cancer stem cells has relied on the expression of specific cell surface antigens such as CD20, CD24, CD34, CD44, CD105, CD117, CD133, CD326, CK7, OCT4, and Sca-1 because of their known expression on endogenous stem cells [20]. These markers have been identified in human cancers, including myeloid leukemia [4], brain tumors [30], breast cancer [1], prostate cancer [6], ovarian cancer [2], liver cancer [34], lung cancer [32], and colon cancer [22].

However, there are a limited number of reports on cancer stem cells in animal cancers. Therefore, we performed this study to identify the cancer stem cells in mouse and canine hepatocellular carcinoma and canine mammary gland tumor using antibodies against CD133, CD44, CK7, and OCT4.

## Materials and Methods

### Materials

Formalin-fixed tumor tissues embedded in paraffin were obtained from the archives of the Department of Pathology, College of Veterinary Medicine, Chungnam National University. Mouse hepatocellular carcinomas were induced by *N*-nitrosodiethylamine (DEN) in female and male db/db mice and male FVB-HBX transgenic mice (Korea Research Institute of Bioscience and Biotechnology, Korea, Tables 1 and 2).

Positive controls for CD44 (Thermo Scientific, USA) and CK7 (Santa Cruz, USA), were formalin-fixed and paraffin-embedded canine tonsils [27, 33]. The positive control for CD133 (Abcam, UK) was an O.C.T 4583 compound (Sakura Finetek Europe B.V., USA.) embedded and cryosectioned mouse kidney [13]. The positive control for OCT4 (Abcam, UK) was formalin-fixed and paraffin-embedded sample of rat brain [21]. Appropriate reference tissue sections were used as

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**Table 1.** Case informations of mammary tumors

| Ages | Sex | Diagnosis |
|---|---|---|
| 7 years | Female | Adenocarcinoma |
| 7 years | Female | Adenocarcinoma |
| 10 years | Female | Adenocarcinoma |
| 10 years | Female | Adenocarcinoma |
| 10 years | Female | Adenocarcinoma |
| 11 years | Female | Adenocarcinoma |
| 11 years | Female | Adenocarcinoma |
| 11 years | Female | Adenocarcinoma |
| 11 years | Female | Adenocarcinoma |
| 12 years | Female | Adenocarcinoma |
| 12 years | Female | Adenocarcinoma |
| 15 years | Female | Adenocarcinoma |
| 8 years | Female | Complex adenocarcinoma |
| 7 years | Female | Mixed tumor/malignant |
| 7 years | Female | Mixed tumor/malignant |
| 7 years | Female | Mixed tumor/malignant |
| 8 years | Female | Mixed tumor/malignant |
| 8 years | Female | Mixed tumor/malignant |
| 9 years | Female | Mixed tumor/malignant |
| 9 years | Female | Mixed tumor/malignant |
| 9 years | Female | Mixed tumor/malignant |
| 11 years | Female | Mixed tumor/malignant |
| 14 years | Female | Mixed tumor/malignant |

positive controls for each antibody (Table 3).

**Immunohistochemistry**

Immunohistochemistry (IHC) was performed using monoclonal antibodies specific for CD44, CD133, OCT4, and CK7 (Table 1). After deparaffinizing and dehydrating the tissue samples, antigen retrieval was performed in a 100 w microwave for about 20 min. Endogenous peroxidase activity was quenched by immersion in 0.5% $H_2O_2$ in methanol for 30 min. The tissue sections were blocked with normal serum for 30 min and then incubated with primary antibodies against each antigen and incubated for 1 h at room temperature. As a negative control, the primary antibody was replaced with PBS. After washing, a biotinylated anti-rat, anti-mouse or anti-rabbit antibody (Vector Laboratories, USA) diluted 1 drop (50 μL) in 10 mL PBS was applied, followed by avidin-biotin-peroxidase complex (ABC kit; Vector Laboratories) according to the manufacturer's instructions. The chromogen used was 3,3'-diaminobenzidine-tetrahydrochloride (Vector Laboratories) 4 drops with $H_2O_2$ 2 drops as substrate in 5 mL distilled water with pH 7.5 buffer 2 drops were added. Tissue sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted.

**Table 3.** Primary antibodies used for immunohistochemistry

| Antibody | Clone | Dilution | Positive control |
|---|---|---|---|
| CD44 | Rat monoclonal | 1 : 200 | Canine tonsil |
| CD133 | Rabbit monoclonal | 1 : 200 | Mouse kidney |
| OCT4 | Rabbit monoclonal | 1 : 100 | Rat brain |
| CK7 | Mouse monoclonal | 1 : 200 | Canine tonsil |

**Table 4.** Summary of CD44, CD133, CK7, and OCT4 expression in cancer

| Antibody | Mammary cancers (n = 23) | Liver cancers (n = 8) |
|---|---|---|
| **CD44** | | |
| Positive | 7 (30%) | 2 (25%) |
| Negative | 16 (70%) | 6 (75%) |
| **CD133** | | |
| Positive | 5 (22%) | 3 (38%) |
| Negative | 18 (78%) | 5 (62%) |
| **CK7** | | |
| Positive | 0 (0%) | 0 (0%) |
| Negative | 23 (100%) | 8 (100%) |
| **OCT4** | | |
| Positive | 0 (0%) | 0 (0%) |
| Negative | 23 (100%) | 8 (100%) |

**Table 2.** Case informations of liver cancers

| Strain | Sex | Treatment | Necropsy | Diagnosis |
|---|---|---|---|---|
| db/db | Female | DEN 20 mg/kg at 2-week-old | 26 weeks after treatment | HCC |
| ob/ob | Male | DEN 20 mg/kg at 2-week-old | 26 weeks after treatment | HCC |
| ob/ob | Male | DEN 20 mg/kg at 2-week-old | 26 weeks after treatment | HCC |
| FVB-HBX Tg | Male | DEN 20 mg/kg at 2-week-old | 26 weeks after treatment | HCC |
| mH-ras | Female | DEN 95 mg/kg at 5-week-old | 8 weeks after treatment | HCC |
| mH-ras | Male | DEN 95 mg/kg at 5-week-old | 26 weeks after treatment | HCC |
| mH-ras | Male | DEN 95 mg/kg at 5-week-old | 26 weeks after treatment | HCC |
| Canine | Female | Spontaneous | | HCC |

HCC: Hepatocellular carcinoma, DEN: *N*-nitrosodiethylamine

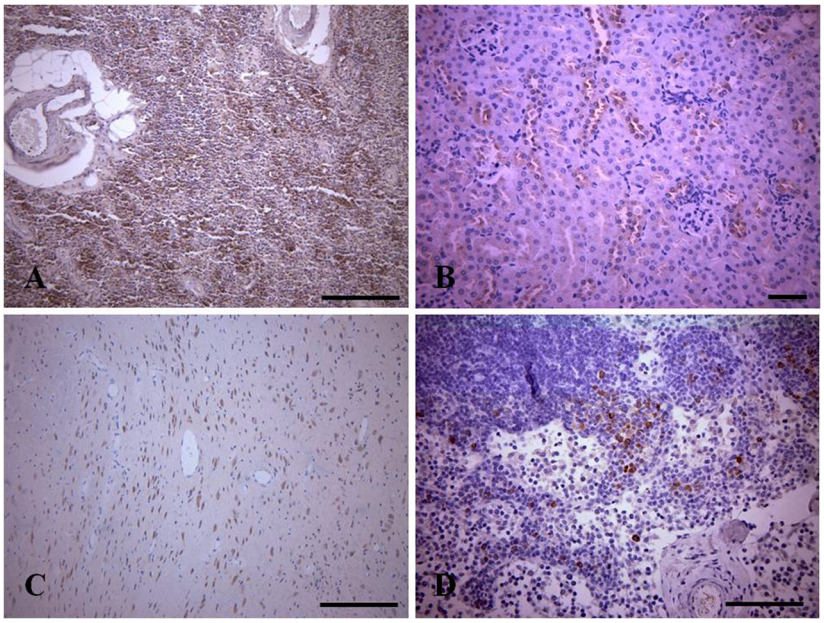


**Fig. 1.** (A) Positive control for CD44 in canine tonsil, (B) Positive control for CD133 in mouse kidney, (C) Positive control for OCT4 in rat brain, (D) Positive control for CK7 in canine tonsil. Immunohistochemistry (IHC), Hematoxylin counterstain, Scale bars = 100 μm.

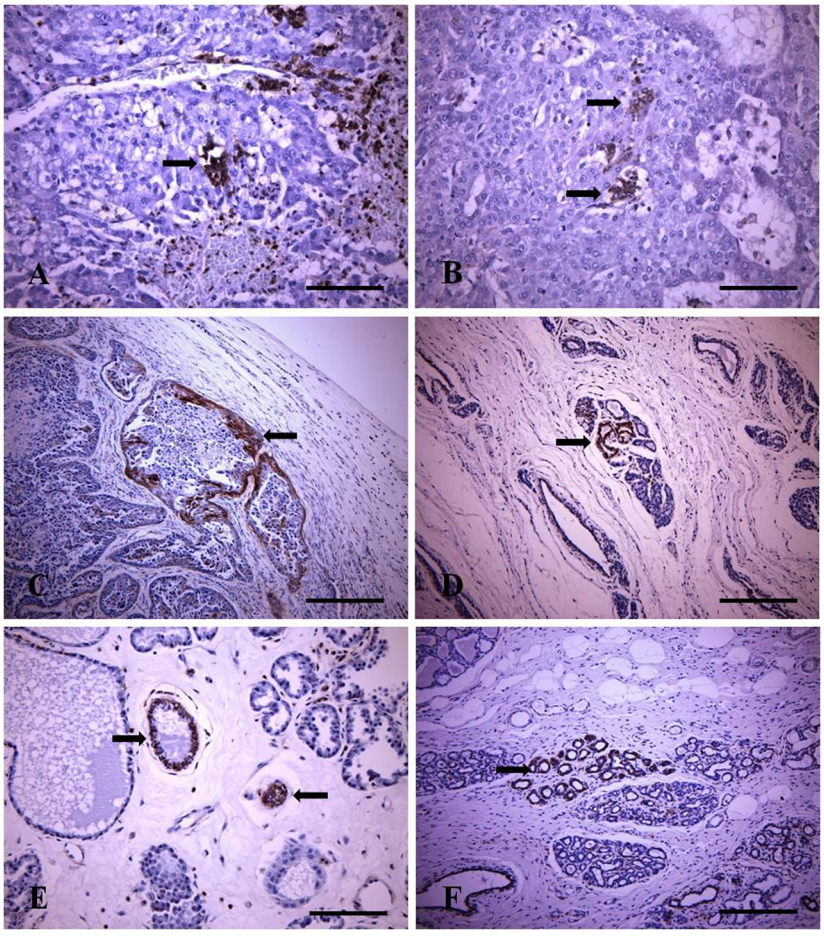


**Fig. 2.** Microphotograph illustrating CD44+ cells (arrows). (A and B) Canine hepatocellular carcinomas, (C) Canine mammary adenocarcinoma, (D and E) Canine mixed tumors of mammary gland, (F) Canine mammary adenocarcinoma. IHC, Hematoxylin counterstain, Scale bars = 100 μm.

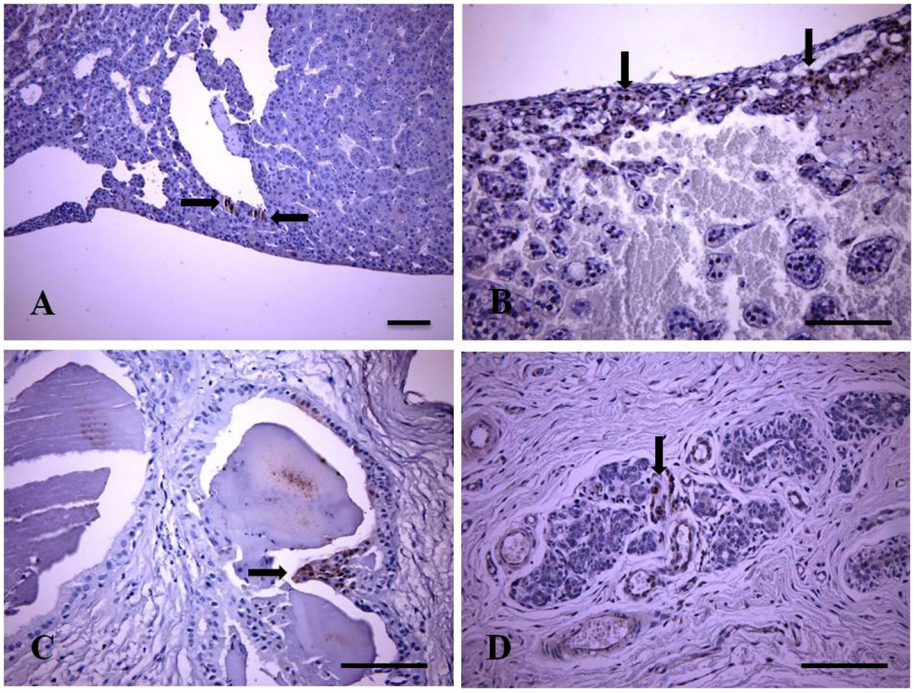


**Fig. 3.** Microphotograph illustrating CD133+ cells (arrows). (A) Mouse hepatocellular carcinoma in male FVB-HBX Tg mouse induced by *N*-nitrosodiethylamine (DEN). (B) Hepatocellular carcinoma in female db/db mouse induced by DEN. (C) Canine mammary mixed tumor, (D) Canine mammary mixed tumor. IHC, Hematoxylin counterstain, Scale bars = 100 μm.

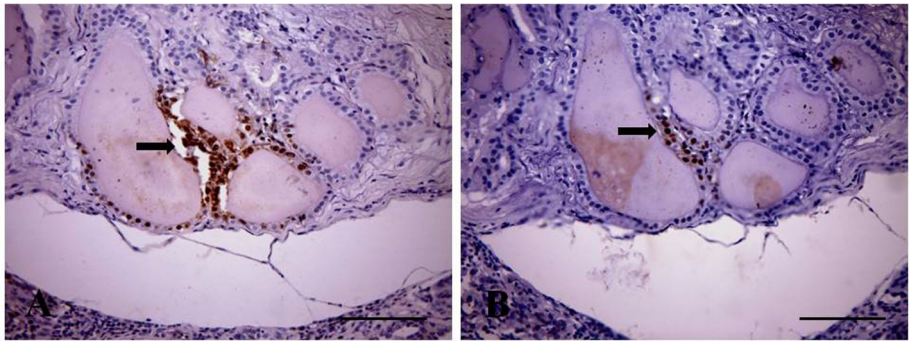


**Fig. 4.** Microphotograph illustrating CD44+ (A) and CD133+ (B) cells (arrows) in canine mammary mixed tumor. (A) CD44 positive, (B) CD133 positive. Hematoxylin counterstain, Scale bars = 100 μm.

## Results

Tumor cell marker expressions were not seen in normal liver and mammary tissues. A total of 23 mammary and eight liver cancers were evaluated. Special control tissues were used in order to test the protocols and for the specificity of the antibodies being used. The purpose of the positive controls was to ensure that the protocol or procedure worked. IHC staining for CD133, CD44, CK7, and OCT4 is shown in Fig. 1. The results are summarized in Table 4.

CD44 was expressed in 30% of the mammary tumors (7/23) and 25% of the liver cancers (2/8). CD44 expression was found in the cytoplasm and, to a lesser extent, in the nucleus. In hepatocellular carcinoma, CD44+ cells were a subpopulation of neoplastic cells. In mammary tumors, CD44+ cells were found to have primarily originated from epithelial cells; this was a rare occurrence in myoepithelial cells (Fig. 2).

CD133 was expressed in 25% of the mammary tumors (5/23) and 38% of the liver cancers (3/8). CD133 expression was observed in the nucleus of alveolar epithelial cells and interlobular duct cells. CD133 was mainly expressed in higher stage tumors and undifferentiated tumors, indicating that CD133 expression is associated with more aggressive tumors. CD133+ cells were also found in mouse hepatocellular carcinoma (Fig. 3). Moreover, CD44 and CD133 were expressed in the concurrent canine mammary mixed tumor tissues (Fig. 4). However, CK7 and OCT4 were not expressed in mammary and liver cancers.

## Discussion

Although the development of antitumor therapies has progressed, most tumor patients still cannot be treated and are prone to tumor recurrence and metastasis [35]. Recently, the role of cancer stem cells in tumor initiation and progression has been the focus of several studies. Searching for specific cancer stem cell markers is essential for further investigating the initiation, invasiveness, differentiation, metastasis, and prognosis of tumors [5, 35].

CD44, CD133, CK7, and OCT4 are the most important cancer stem cell markers. CD44, one of the most widely-recognized cancer stem cell markers, is a transmembrane glycoprotein that mediates cell-cell and cell-matrix interaction though its affinity for hyaluronic acid [12]. CD44 has been described as a marker of breast tumors [1], colorectal cancer [8], head and neck cancer [25], and pancreatic cancer [16]. In the present study, CD44+ cells were a subpopulation of neoplastic cells in canine mammary tumors and murine hepatocellular carcinoma. In canine mammary tumors, CD44+ cells were primarily observed in ductal epithelium or the epithelium of mammary alveoli; these cells were not found in rest of them such as myoepithelial cell or blood vessel. Not expressed at a high rate in our study, but CD44+ cells discovered in papillary carcinoma mostly originated from mammary alveolar epithelial cells or ductal epithelial cells, and rarely formed from myoepithelial cells or fibroblasts [23]. In the present study, CD44+ cells were also found in hepatocellular carcinomas.

CD133 is a pentaspan transmembrane glycoprotein first identified in human hematopoietic stem cells with a monoclonal antibody [19]. CD133 has been used as a surface marker to isolate stem cells from a variety of tissues [15, 28]. It has also been described as a marker for brain tumors [30], colon cancer [22], prostate cancer [6], and malignant melanomas [14]. In addition, CD133 is expressed by a small population of human hepatocellular carcinoma cell lines and primary human hepatocellular carcinomas, and CD133 expression is associated with a high degree of tumorigenicity [17, 34]. Not expressed at a high rate in our study, but CD133+ cells were identified in mouse hepatocellular carcinomas and canine mammary tumors. In addition, CD44 and CD133 were expressed in the same tissues. The findings from canine mixed mammary tumors will need to be substantiated using primary tumor samples before any definitive conclusions can be made about the usefulness of CD44 and CD133 as a cancer stem cell marker in mammary tumors.

Cytokeratin 7 (CK7) reacts with proteins that are found in most ductal, glandular, and transitional epithelium of the urinary tract, and in bile duct epithelial cells. CK7 can be used to distinguish between lung and breast epithelium that are positive, and colon and prostate epithelial cells that are negative. CK7 also reacts with many benign and malignant epithelial lesions such as adenocarcinomas of the lung and liver [32, 34]. OCT4 is a key transcription factor that is necessary for the self-renewal and pluripotency of embryonic stem cells and germ cells [18]. OCT4 is largely expressed in human germ cell tumors and its expression has also been found in several somatic cancers including prostate [31] and bladder cancer [3]. In the present study, CK7 and OCT4 were not expressed in mammary or liver cancers. This may be because tumor cells that have characteristics of cancer stem cells from species may express different phenotypes. In other words, the phenotype of one type of cancer stem cells from a certain species cannot be directly compared to that of cancer stem cells from another species.

In this study, we hypothesized that subpopulations of CD44+, CD133+, CK7+, and OCT4+ cells might represent subpopulation of cancer stem cells or progenitor cells that are responsible for the maintenance and endless proliferative capacity of the respective neoplasms. We considered stem cells in animal cancer with CD44+, CD133+, CK7+, and OCT4+ phenotypes as stem cells in similar to ones found in human cancer. However, according to the data from this study, all animal cancer stem cells do not express CD44, CD133, CK7, or OCT4. Cancer stem cells from different species may express different phenotypes. In other words, one phenotype of cancer stem cells in certain species cannot be applied directly to another species. Cancer stem cells such as CD31 [9], CD34 [9], CK20 [32], CD326 [16], TTF1 [32] etc. had been identified in human myeloid leukemia, breast cancer, prostate cancer, brain tumor, and ovarian cancer [29]. Therefore, other stem cell markers need to be confirmed by further investigation in animal tumors.

The number of cases studied is not clear with respect to the potential for statistical analysis. The markers used to identify cancer stem cells, surely normal tissue of same origin/species should be used as comparison. Other data on the molecular and functional characteristics of the cells, including work in cell culture and tumor transplantation studies, would be necessary to substantiate that conclusion.

Most studies have focused on the identification of tumor cell populations enriched by cells with stem cell-like properties. We have little understanding of the significance of the markers used to identify these cells; whether they are simply markers of convenience or whether they have functional significance remains unknown. These molecules may play a role in tumorigenic, metastatic, and treatment resistance properties of cancer stem cells. Elucidating the role of these molecules is certainly a logical step in discovering for a full molecular understanding of these cells and how they compare to normal stem cells and remaining tumor cell populations. An understanding of cancer stem cells has the potential to identify novel therapeutic targets and impact patient care [20].

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