CHAPTER I

INTRODUCTION

Cancer original develop from normal cells that rise to mutation resulting in the ability to abnormal proliferation and finally turn to malignant. Cancer is basically a disease of regulation of tissue growth caused by abnormalities in the genetic material of the transformed cells(1). These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome. During carcinogenesis, genes which regulate cell growth and differentiation must be altered (2) and are used as molecular signature of cancer cells. In fact that cancer cells are highly heterogeneous. Recent studies suggested that there was small fraction of cancer stem cells the so-called “side population” reside in various types of tumor tissues(3). Most of studies present the small fraction of cancer stem cells that reside in the tumor (4-6). This is the importance problem to limit the success of therapy because cancer stem cells are believed to mediate cancer recurrence after chemotherapy. These authors suggested that cancer growth within a given neoplastic process may be dependent upon only a small fraction of progenitor cells (7, 8). They also suggested that the cancer cells still retain the normal stem cell ability, self-renew and pluripotency are often referred to as cancer stem cells. The first developed of cancer stem cells (CSCs) found in a

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subpopulation of leukemic cells that express a specific surface marker $CD34^{+}/CD38^{-}$ (9). The concept of cancer stem cells was expanded to multiple cancers including solid tumor such as Breast cancer, prostate cancer and colon cancer (10-15). Many studies provided convincing support of the cancer stem cells hypothesis (16-18). The colon adenocarcinoma, is one type of solid tumor that have multiple studies have recently provided confirm cancer stem cells hypothesis as side population cells in the tumor. Colon cancer stem cell was first identified through the expression of cluster of differentiation (CD). The small fraction of cancer stem cell in the colorectal adenocarcinoma could be identified by specific markers, antibody to CD 133. O'Brien and Pollett study in the colon cancer derived from patient showed $CD133^{+}$ in tumor range from 7.5-15.9%(19). The Caco-2 cell line is an epithelial cancer. Throughout the body, epithelial cells form well-ordered sheets that are anchored to basement membranes. These serve as a barrier between the interior of the body and the outside world. The Caco-2 cell line obtained from ATCC is the one type in a group of colorectal adenocarcinoma. The hypothesis of small fraction of cancer stem cells in cancer cell line is the current field will be study. The small population of cancer stem cells is the cause of recurrent cancer through multidrug resistance protein by pumping chemotherapy drugs out of the cells. Interestingly in many stem cells are expressed protein transporters in group of ABC transporter proteins such as breast cancer resistance protein1 (BCRP1). When used of Hoechst 33342, substrate of BCRP1 to identified the subset of stem cells (side population in the cell line). J Burkert and WR Otto verified the present of side population phenotype in seven gastrointestinal cell lines (HT29, HGT101, Col1, Col29, SW620, Caco-2 and HRA19a1.1) in the present of reserpine, ABC-transporter blocking. The result show the side population in cell

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line was 1.29-17% and decrease in the present of reserpine to 0-0.27% (16). It should be note that in cancer cell line has a subset of cancer stem cells know as side population cells which have the properties like normal stem cells, self-renew and divers differentiate. These properties are the basic function in tissue formation.

1.1 The differential of Normal stem cells and cancer stem cells

Stem cells are the class of undifferentiated cells is able to differentiate into specific cell types. Commonly, stem cells come from two main sources

1. Embryonic stem cells are derived from embryos that develop from eggs that have been fertilized *in vitro* then collected from inner cell mass of blastocyst.
2. Adult stem cells are an undifferentiated cells found within differentiated cells in the tissue or organ.

The ability of normal stem cells are Self-renew, differentiate too many specific cell types and homeostasis. The capacity to self-renew of stem cells through cell division in 3 patterns 1. Asymmetric divisions get one stem cell, one progenitor cell. 2 self-renewing symmetric division get two daughter stem cells. 3 non self-renewing symmetric divisions get two progenitor cells. Colon is the one rapidly self-renew organ. The self-renewing of intestinal epithelial was control by crypt unit, the test-tube shaped structures called crypts of Lieberkühn. The colon stem cells reside in the crypt base and maintain the self-renew of epithelial cells through the mechanism as follow stem cells at the crypt base, call crypt base columnar cells that give rise to transit amplifying cells (TA cell/progenitor) that differentiate into the four major epithelial cell types: enterocytes, goblet cells, enteroendocrine cells and paneth cells (14). Thus these mechanisms are maintaining the homeostasis of epithelial cells. In the recent study was confirmed that in the colon cancer also have the colon cancer

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stem cells population. Identified by isolate the cancer cells from tumor and then culture in DMEM/F12 supplemented with several factors then observed sphere-forming cells and sorting by cells surface marker CD133 and fluorescence-activated cell sorting (FACS technique) exhibit CD133 positive 82.0 % (20). From the self-renew property of cancer stem cells thus the researcher groups were suggested that the cancer stem cells originating from normal stem cells or progenitor cells by genetic or epigenetic alterations. Base on the cell division property the difference between normal stem cells and cancer stem cells is all most normal stem cells enter to asymmetric division give rise one stem cell and one progenitor cell to still remain homeostasis. While in the cancer stem cells it loss of the ability of homeostasis. More than fifty percent put into symmetric division to originate two cancer stem cells(13) resulting in loss of ability of homeostasis (figure 1.1). From this cause affect to cancer cell highly proliferated. The small fraction of cancer stem cells that reside in the tumor might be survives from chemotherapy and act as the quiescent cells. This is the current hypothesis of cancer recurrent.

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Figure 1.1 Scheme of division pattern of cancer stem cells that affect to tumorigenic and cause of cancer recurrent after chemotherapy.

1.2 Colon cancer stem cells

In the presently all of adult mammals tissue was identified have the sub populations of stem cells including colon. The epithelial self-renewal of colon intestine regulate by Wnt pathway. Wnt signaling pathway is the protein network has importance role in the embryogenesis and cancer. The name Wnt was coined as a catenation of Wg (wingless) and Int and is pronounced “wint”. In the epithelial self-renewal Wnt pathway regulate cell proliferation when lack of Wnt pathway cause blocks cell proliferation in intestinal crypts and destroy epithelium cells(21). The colon cancer stem cells originated from normal colon stem cells or TA cells/progenitor when abnormal had changed in Wnt pathway. Nowadays the colon cancer stem cells could be identified by specific cell surface markers (cluster of different: CD). The first colon stem cell marker is Musashi 1(Msi-1), an RNA-binding protein (22) for the colon cancer stem cells Msi-1 was expressed also (5). Afterwards the CD133 (prominin 1), a self-renewal tumor angiogenesis was identified as a specific cell surface marker for endothelial cells(23) and expanded to colon cancer epithelial cells (17). Nowadays many researchers interested in the sub population cells, termed “side population” (SP) cells found in cancer cell line. They suggested that the SP cells in cell line as a cancer stem cell population. The study of characterization of a side population in cell line was perform to identify that the side population have the characteristic like stem cells by investigate the expression of cluster of differentiation(CD). Haraguchi and Utsunomiya study in the sixteen cancer cell line from gastrointestinal cell line. In the colorectal cancer types showed percentage of SP fraction mean $\pm$ SD is 0.43$\pm$ 0.07% (24). The SP cells was detected in fifteen cell lines obtained from American type culture collection (ATCC) (25).

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Such the result could indicate that in the cell lines has the small fraction of SP cells, as cancer stem cells. For future study we have tried to be studies in the expression of putative stem cell markers on SP cells. In the determination for the presence of putative stem cell markers in cell lines (HT29, HGT101 and Caco-2). Study SP cells compare with non-SP cells. The Caco-2 cell line exhibit the expression of stem cell markers (CD44 87.5±4.8%, CD133 96.4±3.2, CD177 0.03±0.0%, CD34 0.01±0.0%, BCRP 1.6±0.3%) in side population cells. In non-SP cells the expression of CD44, CD34 and BCRP was increased but CD117 and CD133 was decreased (16). The SP cells that occur in cancer cell line verified as cancer stem cells. Thus the SP cell populations should have the stem cell like properties such as pluripotency. When culture in appropriate condition the SP cells could be differentiate to varies specific cell types.

1.3 Culture systems (two-dimensions and three-dimensions)

In the presently most of study of cancer cell biology used as two-dimensional (2D) culture system which is not represents the cancer cells physiology in the body, including the study of drug permeability. To study the function of cancer stem cells in the past used 2D culture system which is the major constraint that we cannot understand the true biology and physiology of cancer tumor cells. Cause of the physiology of cancer cells in *in vivo* system is the complex and dynamic. Usually in the normal tissue structures compose of cells and supporting structure, as Extracellular matrix (ECM). The behavior of cells depend on their interaction with microenvironment such as cells-cells contact, cells-ECM. The 3D *in vitro* models act as a bridge cross the gap between 2D cell culture models and animal models. In the three-dimensional (3D) culture system the cancer cells give rise to diverse

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morphology and gene expression than in 2D system. The recent study show diverse morphology of breast cancer cell line culture in 3D system. Twenty-five breast cell lines were cultured in 3D could be distinct morphology into four groups, round shape, mass shape, grape-like and stellate shape (26). The different expression of signaling protein of twenty-five cell lines in 3D culture system was analyzed by western blotting. The result show diverse protein expression profile. For example ErbB2 protein highly expression in mass shape and grape-like shape than round and stellate shape (26). These suggest that the different microenvironment affect to the growth behavior, morphology pattern including gene profile expression.

1.4 Objectives

The aims of the study are:

1. To investigate and characterization of the small fraction of cancer stem cells that reside in the Caco-2 cell line.
2. To determine the physical microenvironment affect to the morphology change in stem cells behavior.

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