CHAPTER 1

INTRODUCTION

1. Rationale

Cancer is a class of diseases in which a group of cells exhibit uncontrolled growth, invasion, and sometimes metastasis. Cancer may also be called malignancy, a malignant tumor, or a neoplasm (literally, a new growth). Cancer is a major cause of death and public health problem in many parts of the world. Cancer causes about 13% of all human deaths (1). According to the American Cancer Society, 7.6 million people died from cancer world-wide in 2007 (2). Most of cancers are caused by abnormalities in the genetic material of the transformed cells (3). 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. Cancer is fundamentally a disease of regulation of tissue growth. During carcinogenesis, the expression of genes which regulate cell growth and differentiation must be altered (4). Genetic changes can occur at many levels, by gain or loss of entire chromosomes upto a mutation affecting a single DNA nucleotide. There are two broad categories of genes which are affected by these changes. The first group is oncogenes, which may be normal genes, when these genes were expressed at

inappropriately high levels, or altered genes, they will have novel properties. These signify that both overexpression and alteration of these genes promotes the malignant phenotype of cancer cells. The another group is tumor suppressor genes which play role on inhibiting cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes.

During our long time experiences in dealing with cancer cell biology and cancer cell culture, we remarked that the culture systems consisted of two groups of cells. One was the small cells with spherical shape and aggregated in small group of cells found suspended or attached onto the bottom of culture flasks. The other was well differentiated cells that attached on the bottom of culture flasks. After treatments using anti-cancer drugs, these well differentiated cells were killed, and the type of cell death including apoptosis and necrosis, was dependent upon the mode of action of the molecule studied. However, the small cells with spherical shape remain still alive. Recently, we found that these cancer cells can renew themselves, proliferate and organize a new tissue, when the cells are grown in 3D-nanofibrous scaffold systems. These lead to do the first approximation that our culture system might contain a small fraction of “cancer stem cells”. We and other researchers (5-7) formulate the reasonalle hypothesis that resting embryonic stem cells may reside in adult tissue, and that upon activation these cells, they may acquire the ability to give rise to cancer and preserve the similarities in the biology of normal stem cells (5-7). The challenge in eradicating cancer stem cells will be to firstly identify these cells and then to find unique pathways which may be targeted without harming normal tissue stem cells. Thus, cancer stem cells represent a subpopulation of cells within a tumor which is capable of initiating new tumors following a prolonged period of remission. Presumably this occurs, because cancer stem cells have unique

properties such as longevity, quiescence and self-renewal, similar to normal tissue stem cells. Recently, several laboratories have made progress in the identification of this small subpopulation of highly tumorigenic, presumptive cancer stem cells in leukemia, brain and breast cancers (8). Stem cells, as opposed to differentiated cells, are longliving and are more likely to be the subject of mutations that are necessary for cancer initiation and progression. Furthermore, cancer stem cells, similar to normal tissue stem cells, may exist in a quiescent state for a long time and this quiescence property may make these cells resistant to conventional chemotherapeutic drugs, which only target dividing cells. However, the concept of cancer stem cells is still not universally accepted and an alternative stochastic model has been proposed in which any tumor cell is capable of generating a new tumor given the right microenvironment (9). Understanding the behavior of cancer stem cells should better enable the design of therapies targeted at the short-lived, as well as long-lived cancer stem cells.

Cancer can be treated by various modalities such as surgery, chemotherapy, radiation therapy and immunotherapy. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient. Chemotherapy is a promise choice of cancer treatments for systemic and metastasis cancers and takes advantage of the phenomenon that tumor cells are approximately 5-fold more sensitive than normal cells, which permits the use of therapeutic agents to destroy malignancies. However, during chemotherapy, tumor cells often lose this sensitivity and become no less vulnerable than normal cells. This diminished sensitivity to the original drug also extends to a broad class of other drugs, diverse in their structure and targets. This acquired multidrug resistance (MDR) is a major challenge to successful chemotherapy of malignant tumors. It is well known that

anti-cancer drugs, which cause DNA damage, can induce apoptosis. Moreover, the defective control of apoptosis in treated tumors constitutes a form of drug resistance mechanism. Some anti-cancer drugs preferentially attack mitochondrial DNA in favor of nuclear DNA because of the naked condition of mitochondria DNA, which is devoid of protective histone or non-histone protein that are intimately associated with nuclear DNA. Recent reports have suggested that DNA damage results in perturbation of the inner mitochondrial membrane permeability. It has been postulated that mitochondrial permeability transition (MPT) could be caused by exogenous and endogenous reactive oxygen species (ROS). MPT and subsequent mitochondrial membrane potential ($\Delta\Psi_m$) disruption, is a general feature of cells undergoing apoptosis. The decrease in $\Delta\Psi_m$ precedes cell death, implying that damage to mitochondria is an early event leading to cell death and contributes to apoptosis because of the dramatic decrease in cellular energy supply.

Chemotherapy using cytotoxic or DNA-targeted drugs somehow, also harm to normal cells, which causes a range of adverse effects such as cardiotoxicity hepatotoxicity, nephrotoxicity, immunosuppression and myelosuppression. Cancer treatment using chemotherapeutic agents also generate oxidative stress. Consequently, an enhanced lipid peroxidation, reduction of anti-oxidant vitamins, free radical-trapping capacity in plasma, and a marked reduction of tissue glutathione (GSH) (10) levels are frequently detected during chemotherapy (10-12). The overwhelming production of reactive oxygen species (ROS) damages healthy tissues and is therefore considered to be one of the causes of the toxic side effects of chemotherapeutic agents. In particular, tissue and cells with a high proliferation rate, such as the epithelium of the gastrointestinal tract, the bone marrow, and hair follicles are especially affected by the oxidative insults (13). In

order to overcome the MDR phenomenon and avoid the side effect of cancer chemotherapy, anti-cancer drug discovery has refocused on ‘natural product’ anti-cancer agents. Polyphenolic compounds or polyphenols found in various higher plants are the most important anti-oxidants and considered as functional foods, containing phytochemicals constituents which are not included in traditional nutrients. Natural polyphenols have been demonstrated to exhibit a variety of biological activities including anti-oxidation, anti-carcinogenesis, anti-proliferation, anti-inflammation and apoptotic induction.

2. Objectives of the study

The aims of this work are :

1. To investigate the intracellular targets responsible for apoptosis-inducing activities of Siamois® polyphenols in cancer cells as compared to normal cells.

2. To investigate the effect of Siamois® polyphenols on NFκB-dependent apoptotic and inflammatory pathways in cancer cells.