INTRODUCTION

Cancer refers to diverse group of diseases, characterized by uncontrolled growth of abnormal cells which does not obey the complex rules of architecture and function that govern the usual placement and behavior of cells within a tissue, having the ability to invade nearby tissue and migration to distant sites *(Holland et al., 2006)*.

A biomarker or a biological marker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process or pharmacological responses to therapeutic intervention" *(Atkinson, 2001)*. The last few decades have witnessed major advances in biotechnology, which have contributed to increasing knowledge on cancer biology. Among such advances, is the progress in the field of tumor markers and biomarkers *(El-Bolkainy et al., 2005)*.

In a search of new bone proteins, a protein secreted in vitro in large amounts by a human osteosarcoma cell line was identified *(Johansen et al., 1992)*. The protein was named YKL-40 based on its three NH_2-terminal amino acids; tyrosine (Y), lysine

(K) and leucine (L), and its molecular weight of 40 kDa *(Johansen et al., 1992)*. Based on its amino acid sequence, it has been found that YKL-40 belongs to the glycosyl hydrolase family *(Henrissat and Bairoch, 1993)*.

It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protection of cancer cells from undergoing apoptosis, stimulation of angiogenesis or affecting the intracellular tissue remodeling *(Houston et al., 2003)*. Studies revealed that YKL-40 is expressed in patients with hematological malignancies such as acute myeloid leukemia *(Bergmann et al., 2005)*, as well as several types of solid tumors, such as breast, colon, lung, kidney, thyroid, ovary, prostate, uterus, pancreas and many other solid tumors *(Johansen et al., 2006)*.

AIM OF WORK

The aim of this work was to study the possible role of YKL-40 as a novel biomarker for cancer screening and its prognostic implications in various cancer types.

TUMOR MARKERS

I. Definition of Cancer:

Cancer is a singular word that embraces a vast diversity of diseases that can occur in any organ or system throughout the body. The unique characteristic of cancer is the proliferation of cells of a type different even if so slightly, from the normal complement of the organism. The proliferation may be rapid or slow, and the accumulation of cells may be massive or minimal. The essence of the matter, however, is that aberrant cells, distinct from the ordinary evolution of cell types appear and accumulate *(Holland et al., 2006)*.

Cancer is distinguished from other abnormal cellular growths that lead to benign tumors in its characteristic independence from the restrictions present in normal tissues. Benign tumors expand and compress, but do not attack or invade adjacent tissues. By contrast, accumulated cancer cells make a tissue that ignores the anatomical barriers of adjacent cell membranes and basement membranes. Through chemical and mechanical means, the cancer cell insinuates itself between and into the space of the normal cells, killing them by chemical and physical means. Even though, for example, the placenta in

mammals shows this behavior, there is self limitation in location and in survival of the placental invasion *(Chui, 2006)*.

The cancer cell is partially or absolutely insensitive to such normal constraints and may continue invasiveness indefinitely. The cancer process does not start with a fully invasive cancer cell. A disorder in molecular instructions for protein synthesis is the common precursor lesion. Many distortions in DNA can give rise to abnormal or unbalanced RNA messages, leading to quantitative differences in proteins that result in disordered cellular function *(Holland et al., 2006)*.

In their earliest stages, as proliferating cells accumulate, cancers are almost always symptomatic. Cancers cause symptoms as they advance as a consequence of their mass, ulceration on an epithelial surface or change in function of the affected structure or organ *(Chan and Sell, 2001)*.

Cancer is known to result from multiple genetic abnormalities accumulating over a long period of time *(Nakamura et al., 2004)*. A fundamental charac-teristic of malignant tumors is that the abnormal cell is a heritable one. Normally, during the process of

development, a fetal cell slowly becomes mature and differentiates into many different forms of cells and architecture to produce the normal organs of the human body. Due to some stimuli, either from an internal or from an external source, some of these primitive cells fail to differentiate and subsequently proliferate uncontrollably to produce a malignant cell, which in turn produces more and more malignant cells *(Gosh and Gosh, 1987 and Nakamura et al., 2004)*.

II. Factors affecting cancer:

Most cancers are caused by the combined interaction of genetic factors, lifestyle and environmental factors *(Ames et al., 1995 and McPhee, 1995)*.

A. Genetic factors:

Researchers have made significant advances in the study of mutations in DNA sequences that lead to amplification of oncogenes or to the deletion of tumor suppressor genes encoding regulatory proteins that normally suppress cellular proliferation *(Gordon-Cardo, 1995)*. The mutations in cancer can be either germline mutations, which are inherited and associated with familial cancer syndromes, or can be somatic acquired mutations. The somatic mutations

are sporadic and are the most frequent abnormalities associated with tumor formation and progression *(Gregg and Grody, 1997)*.

Most cancers are the result of multiple events involving multiple genes. Certain genetic mutations precede tumor formation and other mutations evolve during tumor progression. These mutations, together with many other markers could be considered, and also used as biomarkers for cancer *(Burck et al., 1998)*.

B. Environmental factors:

Environmental influences that cause oncogenic mutations include radiation, ultraviolet light exposure and chemical carcinogens. Also, infectious agents, such as the human papilloma virus (HPV) and Epstein-Barr virus (EBV), can promote malignancy through interaction with the host genome *(Mutirangura, 2001 and Ferenczy and Franco, 2002)*.

Early detection of cancer offers the best chance for cure. The goal is to diagnose cancer when a tumor is still small enough to be removed completely through surgery. Unfortunately, most cancers do not produce symptoms until the tumors are either too large to be

removed surgically or cancerous cells already have spread to other tissues *(Burck et al., 1998)*.

III. Cancer Biomarkers:

A cancer biomarker is a property of cancer associated with a clinical or a biological outcome. The term "tumor marker" has traditionally referred to a detectable tumor associated protein or antigen occurring exclusively or more commonly in association with cancer cells compared with the normal tissue of origin. A tumor marker is a substance sometimes found in increased amount in the blood, other body fluids, or tissues that may suggest the presence of a type of cancer. Tumor markers are found in cells, tissues, or body fluids. They are measured qualitatively or quantitatively by chemical, immunological or molecular biological methods to identify the presence of cancer *(Sindransky, 2002)*.

A. Historical background of tumor markers:

The first tumor marker was the Bence-Jones protein, which usually indicates the presence of multiple myeloma. Since the use of the Bence-Jones protein as an indicator for the presence of plasma cell malignancy, hormones, enzymes, isoenzymes and

proteins were demonstrated to be tumor markers. Occasionally, such markers were useful in the diagnosis of individual tumors, but the general application of tumor markers for monitoring of cancer patients did not begin until the discovery of alpha feto-protein (AFP) in 1963 and carcinoembryonic antigen (CEA) in 1965 *(Bonfrer, 1990)*.

The production of such markers during fetal development, as well as in tumors, led to the use of the term oncodevelopmental markers and oncofetal antigens. In 1975, the development of monoclonal antibodies for immunoassay led to the discovery of many oncofetal antigens and antigens derived from tumor cell lines, such as carbohydrate antigens. Finally, the advances in molecular genetics, using nucleic acid probes and monoclonal antibodies, led to the discovery of oncogenes and tumor suppressor genes, some of which have proven to be useful tumor biomarkers *(Chan and Sell, 2001)*.

B. The origin of tumor markers:

Morphologically, cancer tissue is recognized as resembling fetal tissue more than normal adult differentiated tissue. Tumors are graded according to their degree of differentiation as being well

differentiated, poorly differentiated, or anaplastic (that is to say without form). Tumor markers are the biochemical or immunological counterparts of the differentiation state of the tumor. In general, tumor markers represent the re-expression of substances produced normally by embryogenically related tissues *(Chan and Sell, 2001)*.

C. Biological functions of Tumor markers:

1. Risk biomarkers:

A risk biomarker conveys the earliest evidence of the probability of developing cancer in subjects who are currently without the evidence of disease. Inherited genetic abnormalities are the most extensively studied risk markers, such as BRCA1/2 mutations in breast and ovarian cancer, and the adenomatous polyposis coli and mismatch repair gene mutations, such as hMSH2, hMLH1 and hPMS2, HMSH3 and hMSH6 in colonic cancer. The presence of a risk marker indicates that cancer is more likely to occur within a defined time period in subjects with the marker compared to the general population *(Srinivas et al., 2001)*.

2. Cancer screening biomarkers:

Conversely, cancer screening markers distinguish asymptomatic individuals at sufficient high risk of cancer to justify the use of additional studies to identify previously undetected malignant disease. Screening markers attempt to detect malignant and pre-malignant disorders at an early stage, such that clinical intervention can be effective *(Mera, 1995)*. Since screening marker tests are intended for use in asymptomatic populations, and the proportion of affected persons is likely to be small, an effective screening marker should exhibit both, high sensitivity and high specificity for malignancy *(Chui, 2002)*.

Such a screening marker would therefore be unlikely to miss a case of disease (a sensitivity issue) or lead to a false positive result in individuals without the disease (a specificity issue). Ultimately, the broad application of a cancer early detection marker is contingent on improvements in long-term outcomes within a population *(Chui, 2006)*.

Screening mammography for breast cancer, the Papanicolaou (PAP) cell cytology test for cervical cancer and the fecal occult blood testing for colorectal cancer, have all been demonstrated to reduce cancer

specific mortality when applied to the general population *(Srinivas et al., 2001)*. On the other hand, considerable uncertainty exists regarding the quantitative cancer related mortality impact of measuring cancer antigen (CA 125) as a screening test for ovarian carcinoma and carcino embryonic antigen (CEA) for gastro-intestinal malignancies and performing chest radiography and/or chest computed tomography for lung cancer *(Chui, 2002)*.

3. Diagnostic markers:

Due to the lack of sensitivity and specificity, measurement of serum tumor markers is rarely of use in the early diagnosis of cancer. Histopathologic analysis of primary tumor tissue continues to remain the gold standard for the diagnosis of malignancy. However, diagnostic tumor markers can provide important information to aid in:

a. Distinguishing between benign and malignant disease.

b. Identifying the primary site in malignancy of an unclear primary origin.

c. Clarifying issues or distinguishing tumor subtypes in difficult or rare cases that

conventional histopathology can not further classify.

(Sindransky, 2002)

4. Prognostic markers:

Prognostic markers are used to predict outcome of the disease such as relapse-free survival, overall survival, and time to disease progression. Traditionally, clinical and histopathlogic parameters (for example tumor size, grade, lymph node status) have been used to assess the prognosis of solid tumors *(Duffy, 1999)*. However, the variability of clinical outcomes within groups of patients classified using these standard parameters undergoes the limitations of current staging classifications to predict the behavior of tumors over time. Molecular analyses of tumor or serum specimens have the potential to describe and/or reflect on the biologic functions of a particular cancer. In breast cancer, estrogen receptors (ER) serve as an important cancer prognostic marker, with longer disease free survival and overall survival observed among patients with ER positive tumors compared with those with ER negative ones *(Anderson, 2001)*.

5. Therapy selection and response markers:

Therapy selection and response markers predict in advance the likelihood of a patient responding to, and therefore benefiting from a specific therapy. For instance, the detection of estrogen receptors (ER) and progesterone receptors (PR) status is useful to predict the response of breast cancer to hormone therapy. Up to 70% of ER and PR positive advanced breast tumors respond to hormone therapies, whereas less than 5% of ER and PR negative tumors respond *(Chan and Sell, 2001)*.

Elevated expression of HER2 (c-erbB-2), as measured by immunohistochemical staining and fluorescence in situ hybridization (FISH) of the HER-2 gene, also predict increased responsiveness of breast tumors to anthracycline chemotherapy versus cyclo-phosphamide, fluorouracil and methotrexate *(Pegram et al., 1998)*. These observations have led the American Society of Clinical Oncology Panel on Breast Cancer Markers to recommend that ER/PR status and HER-2 expression levels are determined for all primary breast cancers at the time of diagnosis *(Sidransky, 2002)*.

6. Recurrence and metastasis markers:

Tumor markers are commonly measured by clinicians in the follow up and monitoring of patients with previously diagnosed and treated malignancies. It is important to recognize that the routine use of recurrence markers depends on demonstrating that early detection and diagnosis would result in the provision of effective therapies that would ultimately improve significant health outcomes (such as overall survival, disease free survival, quality of life, lesser toxicity and cost effectiveness) *(Chui, 2006)*.

Data are insufficient to demonstrate clinical benefit for the standard use of CA 27.29 and carcinoembryonic antigen (CEA) to detect and/or diagnose breast and colorectal cancer metastases respectively *(Bast et al., 2001)*. Similarly, there are major challenges to the use and interpretation of serial prostate specific antigen (PSA) and CA 125 data to monitor for the recurrence and the progression of prostate and ovarian cancers respectively. These diseases may progress without an increase in the levels of biochemical markers *(Kerbrat et al., 2001; Scher, 1999)*.

Conversely, detection of rising serum levels of alpha feto-protein (AFP) and/or the beta subunit of the