I. INTRODUCTION

At present, cancer is one of the most important public health concerns in Thailand and worldwide (http:// www.iarc.fr/cgi-bin/exe/globom.exe). In this study, we focused on colorectal cancers, liver cancer, and lung cancer as they are the most common types of cancer diseases. According to the report from the American Cancer Society, colorectal cancer is the third most common cancer found in men and women, it is estimated that there will be about 106,370 new cases of colon cancer and 40,570 new cases of rectal cancer and they will cause about 56,730 deaths every year. Liver cancer and lung cancer were also the leading cause of cancer death among many types of cancer, accounting for around 14,270 and 160,440 cases each year, respectively (http:// www.cancer.org/docroot/CRI) The death of cancer patients is caused by the ability of cancer cells to invade other tissues and to spread to other parts of the body where they can generate new tumors (Tannock, 1992). Cancer cells require food, oxygen and growth factors in order to grow and spread; therefore, the transportation of these factors to cancer cells is critical for their progression. Since these essential nutrients are transported to the cancer cells by blood vessels, the process that allows new tumor blood vessel formation is thus important and this process is known as "Angiogenesis".

Angiogenesis is a complex process by which new blood vessels arise from the pre-existing vasculature (Folkman, 1997) to transport oxygen and food into cancer cell. In addition to its role in tumor angiogenesis, it also occurs normally in the body at specific times, such as; embryonic development, wound healing, the female reproductive cycle and the growth of bone. However, its dysregulation contributes to several pathological conditions such as diabetic retinopathy, rheumatoid arthritis and the development of solid tumors (Folkman, 1990; Folkman and Hanahan, 1991). The switch from the normal quiescent vasculature to angiogenesis is induced by factors released predominantly by surrounding pericytes and lymphocytes. Such angiogenic factors include acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF) and thymidine phosphorylase (TP), which are directly angiogenic, as well as transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α), which act indirectly. However, the only growth factor that is observed almost ubiquitously at sites of

angiogenesis and whose levels correlate most closely with the spatial and temporal events of blood vessel growth is vascular endothelial growth factor (VEGF).

VEGF is the angiogenic factor which able to induce endothelial cell proliferation and migration. However, VEGF has several other pro-angiogenic activities. It induces endothelial expression of proteases such as interstitial collagenase and the urokinase-type and tissue-type plasminogen activators (uPA and tPA) (Pepper *et al.*, 1991; Unemori *et al.*, 1992). These proteases release cells from anchorage, allowing migration, and can generate by products that affect angiogenesis. VEGF also stimulates microvascular leakage (Senger *et al.*, 1983; Connolly *et al.*, 1989; Keck *et al.*, 1989) and hexose transport (Pekala *et al.*, 1990). In addition VEGF participates in the continued survival of nascent endothelial cells (Alon *et al.*, 1995; Benjamin and Keshet, 1997).

Most types of tumor overexpress VEGF mRNA. This expression directly correlates with regions of angiogenesis and high vascular density (Ferrara and Davis-Smyth, 1997). High levels of VEGF are generally associated with hypoxia, an excess of soluble inducing factors or unregulated VEGF expression. Vascular endothelial cells in the tumor vicinity also appear to upregulate expression of VEGF receptors. The newly formed blood vessels are inherently leaky, which enhances the likelihood of metastasis. Studies relating VEGF expression to tumor aggressiveness, metastatic potential and the probability of relapse indicated that high levels of VEGF expression correlated with poor prognosis (Toi *et al*, 1994; Maeda *et al*, 1996). Because of this characteristic, VEGF has been associated with tumor growth, invasion, and metastasis in solid tumors (Folkman, 1995; Ellis *et al.*, 1998; Leung *et al*, 1989; Park *et al*, 1993; Poltorak *et al*, 1997; Tischer *et al*, 1989).

The expression of VEGF is subject to complex regulation as alternative splicing of the human *VEGF* gene results in at least six isoforms containing 121, 145, 165, 183, 189, and 206 amino acids (Leung *et al*, 1989; Tischer *et al*, 1991; Houck *et al*, 1991; Poltorak *et al*, 1997; Lei *et al*, 1998), because of differential incorporation of the basic residues encoded by exons 6 and 7. VEGF isoforms differ in their heparin-binding properties, membrane association, and secretion. $VEGF_{121}$, which lacks the basic residues of both exons, does not bind heparin-containing cell surface proteoglycans, and is freely soluble. $VEGF_{165}$ is also secreted; however, cationic residues in exon 7 enable it to bind heparin, and, thus, some remains bound to the cell

surface or extracellular matrix (Park *et al.*, 1993). The large isoforms, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$, which retains both exons, has the highest affinity for heparin and, therefore, remains tightly cell associated.

Detection of circulating VEGF has long been known as a potential serum diagnostic marker for malignant disease. Kondo et al (1994) first recognized the potential of VEGF as a serum diagnostic marker for malignant diseases. Increased serum concentrations of free VEGF have indeed been measured in various types of cancer, including brain, lung, gastrointestinal, hepatobiliary, renal, ovarian (Jelkmann, 2001). However, understanding of the relationship between the pattern of the production of VEGF protein isoforms in tumor tissues and its concentration in the circulation is still insufficient.

A number of studies have shown that expression of certain VEGF transcripts are correlated with tumor progression. Some reported that increased mRNA expression of $VEGF_{189}$ is correlated with poor prognosis in colon cancer (Oshika *et al.*, 1998), esophageal cancer (Tokunaga *et al.*, 1998), and non-small cell lung cancer (Oshika *et al.*, 1998). Whereas others found that expression of $VEGF_{121}$ was correlated with lymph node metastasis in primary lung tumors (Ohta *et al.*, 1997). However, the $VEGF_{165}$ isoform was demonstrated to be the most prominent isoform in promoting vascular density of the implanted tumors in nude mice, whereas $VEGF_{121}$ only partially rescued tumor growth and $VEGF_{189}$ failed completely to promote tumor expansion (Grunstein *et al.*, 2000). Although increased of certain VEGF transcript has been demonstrated to correlate with the progression of various tumors, the actual protein level of the different VEGF isoform and their significance during cellular transformation is unknown. Moreover, it has been suggested that elevated protein expression in tumor tissues was mediated by both enhanced transcription and translation (Scott *et al.*, 1998). In order to understand the role of VEGF in tumor progression, thus it is important to investigate expression of different VEGF isoform at the protein level during tumorigenesis. In addition, no studies focusing on the VEGF isoform pattern at protein level and their relation with respect to total VEGF in circulation has been reported to our knowledge.

Aims of the study

1. The protein expression pattern of VEGF isoforms in colorectal, lung and liver tumors in comparison to the corresponding adjacent normal tissues in order to understand whether which VEGF protein isoforms play an important role during tumorigenesis.
2. The relationship between the expression pattern of VEGF in tumor tissues and level of total circulating VEGF in the blood.
3. Comparing level of circulating VEGF in patients and healthy volunteers in order to investigate whether circulating VEGF can be potentially used as a tumor marker.