DISCUSSION

It has become clear that the growth of solid tumor is dependent on the process of angiogenesis and that VEGF is a central positive regulator of this process. In this study, we described the expression pattern VEGF isoform in tumor tissue of colorectal, liver and lung in comparison to normal tissues of each organ, respectively. Three major protein bands with molecular weight 18, 23 and 26 kDa were predominately detected. The 23 kDa protein band is believed to be the $VEGF_{165}$ as this band was at the same position as the recombinant $VEGF_{165}$ protein standard used in this study. Therefore, protein band with molecular weight 18 and 26 kDa are assumed to be $VEGF_{121}$ and $VEGF_{189}$, respectively.

In colorectal tumors, it was found that $VEGF_{121}$ was equally expressed in both tumor and normal tissues, whereas the $VEGF_{165}$ and $VEGF_{189}$ were only detected or detected at higher level in tumor tissues (Figure 10a). However, in lung tumor $VEGF_{121}$ appeared to be predominately expressed in normal tissues, where as $VEGF_{165}$ and $VEGF_{189}$ were predominately expressed in tumors tissues (Figure 10c). Unexpectedly, it appeared that while tumor tissues of colorectal and lung expressed high level of VEGF isoforms in comparison to normal tissues, normal tissues of liver expressed higher level of VEGF compared to tumor tissue (Figure 10b). Although, more information needed to be gathered, it is possible that regulation of VEGF expression in liver tissues may be different from other types of tissue.

Expression of $VEGF_{165}$ was significantly correlated with a tumor size smaller, whereas $VEGF_{189}$ was significantly correlated with advanced clinical stage of the tumors. Although no significant correlation between expression of the VEGF isoform and clinical stage of the patients was observed in liver and lung tumors, this may due to the fact that only 18 liver tumors and 20 lung tumors were investigated in this study. However, we found that VEGF isoform with molecular weight 23 and 26 kDa are predominately expressed in these two types of tumors with moderately or poorly differentiation (Table 8 and Table 9), although it was not statistically significant.

Our result in colorectal tumors is consistent with the previous reports where expression of $VEGF_{189}$ mRNA has been shown to be correlated with poor prognosis in colon cancer,

esophageal cancer and non-small cell lung cancer (Tokunaga *et al.*, 1998a, Tokunaga *et al.*, 1998b, Yuan *et al.*, 2001). In addition, it has also been demonstrated that $VEGF_{189}$ was expressed at high level in colorectal cancer patient with liver metastasis, indicating its important role in tumor progression (Tokunaga, 1998a).

$VEGF_{165}$ has also been demonstrated to play important role in tumorigenesis. When different isoforms of VEGF were transfected into the VEGF-null cells in isolation and the transfected cells were implanted into nude mice, it was found that $VEGF_{165}$ was the most prominent isoform can fully rescue tumor expansion, while $VEGF_{121}$ and $VEGF_{189}$ only partially or failed completely to rescue tumor growth, respectively. In this study we found that although $VEGF_{165}$ was predominately expressed in colorectal tumor tissues in comparison to normal tissues, its expression was significantly correlated with smaller size (maximum diameter less than 5 cm.) of the tumors (Table 7). Therefore, it is possible smaller size of tumors try to expand themselves by increase the production of $VEGF_{165}$. However, when these tumors become larger, they stop producing $VEGF_{165}$.

Although expression of $VEGF_{121}$ mRNA has been previously reported to be correlated with lymph node metastasis (Tokunaga *et al.*, 1998a), in our study we found that level of the 18 kDa VEGF, which believed to be $VEGF_{121}$ was equally expressed in both normal and tumor colorectal tissues and predominately expressed in normal tissues of lung.

The measurement of total VEGF showed that levels of total VEGF protein were significantly different in tumor tissues compared to normal tissues. It is possible that during tumorigenesis, tumor cells induce the expression of certain isoforms of VEGF to help with their progression. This may explain why we observed the alteration of VEGF expression pattern and also the increase total VEGF in tumor tissues of colorectal and lung. However, we did not observe any different between total level of VEGF in tumor tissues and normal tissue of lung, this may be because normal liver tissues already express quite a high level of VEGF.

It is commonly known that VEGF exists as both soluble and cell-associated isoforms (Park *et al.*, 1993). 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, dose 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 larger isoform $VEGF_{189}$ which retains both exon, have the highest affinity for heparin and; therefore, remained tightly cell associated (Park et al., 1993).

Detection of VEGF has long been known as a potential serum diagnostic marker for malignant disease. Increased serum VEGF concentrations have indeed been measured in various types of cancer, including, brain, lung, gastrointestinal, hepatobiliary, renal and ovarian (Kondo *et al.*, 1995). However, understanding of the relationship between the pattern of the production of VEGF protein isoform in tumor and its concentration in the circulation is still insufficient. In this study, we determined expression pattern of VEGF isoform in tumor tissue in relation to level of total VEGF in patient's serum. This may due to the fact that $VEGF_{121}$ isoform appeared not to be differently expressed in colorectal tumor tissues compared to normal tissues; therefore, $VEGF_{121}$ from normal tissues may also be secreted into the circulation as well as those from tumor tissues.

The comparison of the VEGF level in serum of cancer patients compare to normal volunteers revealed that cancer patients possessed significantly ($p<0.001$) higher level of serum VEGF than those in normal control. While normal control possessed only 605 ± 384 pg/ml serum VEGF, they were 1068 ± 649, 1251 ± 568, and 836 ± 346 in colorectal, lung and liver cancer patients, respectively. However, we found that it's very difficult to decide the cut-off value, as some normal volunteers also possessed quite a high level of VEGF, which may due to the possibility that normal tissue can also produce $VEGF_{121}$ that is secretable into the circulation.

Level of circulating VEGF has been demonstrated to have prognostic value in cancer patient (Kondo *et al.*, 1994). One study performed in colorectal cancer has compared prognostic value of serum VEGF to CEA and CA19-9, tumor markers for gastrointestinal tumor. It was found that serum level of VEGF, CEA and CA19-9 was all correlated with tumor stage and shorter of the rate of survival of the patients. However, only serum level of VEGF was found to have an implication for predicting the response of cancer patient to chemotherapy. Response to chemotherapy was significantly higher in patients with low VEGF level than those with high VEGF level (Hyodo *et al.*, 1998). Therefore, it remains to be further investigated whether circulating level of VEGF will have any predictive value for response to cheomotherapy in Thai cancer patients.

However, circulating VEGF may also be increased in other physiologic and pathologic conditions, i.e., during pregnancy and in response to hypoxia and inflammation. For example, ischemia of the heart produced an acute increase in serum free $VEGF_{121/165}$ concentrations (Seko *et al.*, 1997). Moreover, the levels of circulating VEGF were also increased in rheumatoid arthritis (RA) and its level was significantly correlated with radiographic progression of disease over the subsequent year (Taylor, 2002). The significant reductions in serum VEGF concentrations following response to therapy in RA suggest that angiogenesis is an important process in perpetuating synovitis and raises the possibility that persistence of joint inflammation indicates an imbalance between inducers and inhibitors of angiogenesis.

In this study, we found that although total VEGF level was significantly increased in cancer patients in comparison to normal volunteers, levels of circulating VEGF were not significant correlated with any of the pathological features. In addition, we found that it is very difficult to decide the cut-off value as some healthy volunteers also possesses quite a high level of circulating VEGF. This may be due to the following possibilities: 1) The VEGF isoform that appeared to be significantly correlated with tumor progression is $VEGF_{189}$, which is the cell-associated isoform, not secretable. 2) Some normal tissues, i.e., lung and liver tissues expressed high-level VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$) that can be secreted into the circulating. 3) Expression of some secretable VEGF ($VEGF_{165}$) was negatively correlated with the progression of tumor size. 4) Other physiologic and pathologic condition, i.e., pregnancy, RA and cardiovascular diseases can also cause in the induction the circulating level of VEGF. Although more investigations are needed before any conclusion can be drawn, measuring circulating level of VEGF may have limited used as a tumor markers.

However, it remains to be elucidated whether level of total circulating VEGF have any predictive value for rate of survival of cancer patient. In addition, further study is needed in order to evaluated whether measuring specific $VEGF_{165}$ rather than total VEGF in serum will have any prognostic value.