血管新生在肿瘤生长及转移上扮演重要角色。转移过程中癌细胞必需进入血管中，随血流运至远处器官，离开血管并植入组织中，癌细胞逐步增生，形成小肿瘤；再引发局部肿瘤血管新生；转移之癌细胞可再经由相同方式转移至其它器官。

血管新生是一种复杂过程，包括内皮细胞分解基底膜、内皮细胞侵入间质、内皮细胞增生及移行，最后组成管状之微血管结构。此过程受到二种分子之调节，即是血管新生因子及血管新生抑制因子。

血管新生因子包括血管新生因子家族；血管新生抑制因子包括血管新生抑制因子家族。血管新生之过程，除了...外，最近又发现另一对血管内皮细胞之血管新生抑制因子家族，包括一...的蛋白，可经由具血管内皮细胞之...及抑制血管内皮细胞之增生、移行以及形成微血管新枝，以形成成熟之微血管结构并调节血管之功能。
Angiogenesis plays an important role for cancer growth and metastasis. Cancer cells have to enter the systemic circulation to metastasize to target organs. The degree of angiogenesis induced by tumor was reported to correlate well to the advancement and systemic metastasis in breast cancer, prostate cancer, lung cancer and a variety of malignant tumors.

Angiogenesis is a complicate process, which involves degradation of basement membrane by endothelial cell, invasion of endothelial cell to the stroma, endothelial cell proliferation and migration, and finally organization of proliferated endothelial cell into capillary structure. This process is under control of local activity of two kinds of molecules: angiogenic factors and angiogenesis inhibitors.

The angiogenic factors include bFGF, aFGF, vascular endothelial growth factor (VEGF) and other growth factors. In contrast to angiogenic factors, the angiogenesis inhibitor inhibit the process of angiogenesis. There are 15 currently identified natural inhibitors of angiogenesis and among them, thrombospondin-1 and angiostatin show specificity for endothelia.

Recently, another endothelium-specific angiogenic factor family has been found, that is angiopoietins. Angiopoietin-1 (Ang-1), a 70 KD protein, is a ligand for an endothelium-specific receptor tyrosine kinase- Tie2 receptor, and can signal through this receptor to regulate the angiogenesis process and
vessel remodeling. Angiopoietin-1 is able to recruit and sustain peri-endothelium supporting cells (i.e. pericyte) and to enhance the interaction between endothelium cell and surrounding supporting cell so as to form a mature capillary network, and to modulate the function of blood vessels. Angiopoietin-2 (Ang-2), with 60% homology to angiopoietin-1, can also bind the Tie2 receptor on endothelium. However, Ang-2 can inhibit the interaction between endothelium cell and surrounding supporting cell, loosen the matrix contacts, to facilitate the endothelium proliferation and capillary sprouting. Ang-2 is selectively and highly expressed in ovary, uterus, and placenta, where the angiogenesis and vessel remodeling process is active. Although the Ang-2 is a natural antagonist to Ang-1 for Tie2 receptor on endothelium, both play an important role in the processes of angiogenesis and vessel remodeling.

Tie2, Ang-1 or Ang-2 knockout mice die later in embryogenesis (E 9.5~E10.5). The Tie2, Ang-1 or Ang-2 null phenotype showed the vessels are immature, lacking branching networks and proper organization into large and small vessels. There was an absence of angiogenesis in neuroectoderm and in other organ (kidney). In the cornea micropocket assay model of neovascularization, the Ang-1 was shown to increase the perfusion of VEGF-induced neovascularization, and Ang-2 was shown to increase the length and number of these new vessels.

The complete sequence of Ang-1 and Ang-2 mRNA has been reported in the GenBank, and the localization of Ang-1 and Ang-2 gene has also been reported in literature. This makes the detection of Ang-1 and Ang-2 mRNA possible by the polymerase chain reaction.

The roles of Ang-1 and Ang-2 in the tumor angiogenesis is still unknown, and rarely been investigated. In the previous studies, we investigated the relationship of angiogenic factor (VEGF) and angiogenesis inhibitor (TSP-1) expression to the angiogenesis in non-small cell lung cancer. In this project, we will evaluate the Ang-1 and Ang-2 expression in lung cancer specimens by immunohistochemical staining and by quantitative reverse transcription polymerase chain reaction (RT-PCR). The Ang-1 and Ang-2 expression will be also correlated to the microvessel counts in the lung cancer specimen.

**Keywords:** Angiopoietin-1, angiopoietin-2, angiogenesis, lung cancer, angiogenic factor

二、緣由與目的

Lung cancer is the most common cause of cancer-related death in Taiwan and other industrialized nations and is a significant public health problem(1). While the incidence of many other malignancies has declined or remain stable, the incidence of lung cancer is expected to continue to increase into 21st century, with a predicted mortality rate of 523 deaths per 100,000 population in the 1990s(2). The prognosis of lung cancer is poor as compared with other malignancies, and the five year survival rate
was less than 15% under current form of therapy (2).

The pathobiology of lung cancer is complex, involving oncogenesis, tumorigenesis, evasion of host defense, invasion and metastasis (3). However, the tumor growth and systemic metastasis of all solid tumor depend on neovascularization. Angiogenesis, in which new capillary sprout from existing vessels, is required for tumor growth and metastasis. Angiogenesis not only provides the nourish vessels for tumor growth, but also offers a route for cancer cell to enter systemic circulation to metastasize (4). Folkman showed that the vascular mass of normal tissue is approximately 20%, whereas, during tumorigenesis tumor vascular mass may be 50% of the total tumor (5). In human cancer specimen (breast cancer and cervical cancer), the angiogenesis is also shown to occur in the premalignant stages of these cancers (6-8). These findings suggest that angiogenic activity is both a marker of preneoplastic-to-neoplastic transformation and an event that perpetuates tumorigenesis (9-11). The intensity of angiogenesis of the primary tumor had been reported to correlate well with the regional lymph node and systemic metastasis and prognosis in melanoma, breast cancer and variant malignancies (4,8,9,11-13). In our previous study, the microvessels density was also shown to correlate with the advancement and metastasis in non-small cell lung cancer (14).

The angiogenesis process is complex. At first, the endothelium cell degrades the basement membrane, and invades to the surrounding stroma; then the endothelial cells proliferate and migrate, and change shape and adhere tightly to each other to form a lumen of a new capillary tube. Finally, sprouting tubes fuse and coalesce into loops, circulating the blood into this newly vascularized region (10). This process is under specific regulatory control. The delicate physiological control of angiogenesis depends on the relative local activities of two kinds of molecules: the inducers and the inhibitors (15). For tumor, the angiogenesis is dysregulated in such a manner that a biologic imbalance exists that favors either the overexpression of local angiogenic factors or the suppression of endogenous angiostatic factors (9,11). The inducer (angiogenic factors) includes bFGF, VEGF and other growth factors (16-25). There are 15 currently identified natural inhibitors of angiogenesis and among them, thrombospondin-1 and angiostatin show specificity for endothelial cell (26,27).

The VEGF is endothelium specific, and its expression was shown to increase in hypervascular malignant tumor, such as renal cell carcinoma and liver metastasis of colon cancer (28,29). In our previous study, we also showed the expression of VEGF in non-small cell lung cancers by immunohistochemistry and quantitative PCR. VEGF bind to the VEGF receptor tyrosine kinase (VEGFR) on endothelium. Another endothelium-specific receptor tyrosine kinase family and their
ligand had recently been cloned and isolated, that is Tie2 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains) and angiopoietins (30). Angiopoietin-1 (Ang-1), a 70 KD secreted protein, is not an endothelium mitogen by itself, but is involved in the reciprocal interactions between endothelial cells and the surrounding mesenchyme that are required for new capillary sprouting and remodeling. Ang-1 can recruit and sustain periendothelial support cell (pericyte), and increase the interaction between supporting cell and endothelial cell so as to form a mature capillary network, and to modulate the function of blood vessels.

Angiopoietin-2, like Ang-1, is a ligand to Tie2 receptor on endothelium, and had protein sequence homology to the Ang-1 (60%). Both contain coiled-coil and a fibrinogen-like domain, and bind to Tie 2 receptor with similar affinity. However, the Ang-2 had distinctive effect on the Tie 2 receptor. Ang-1 induces autophosphorylation of Tie2 receptor in cultured endothelial cells. In contrast, Ang-2 does not induce receptor phosphorylation. Rather, it can competitively inhibit Ang-1duced kinase activation of the Tie 2 receptor. Ang-2 blocks the Ang-1/Tie2 signal, resulting in a loosening of this tight vascular structure and thereby exposing the endothelial cells to activating signals from angiogenesis inducers (like VEGF), and allow the endothelial cell to proliferate, migrate and form a vessel sprouting. Therefore, the Ang-2 plays an important role in the vascular remodeling and angiogenesis process. Ang-2 is selectively and highly expressed in ovary, uterus, and placenta, where the angiogenesis and vessel remodeling process is active. Although the Ang-2 is a natural antagonist to Ang-1 for Tie2 receptor on endothelium, both play an important role in the processes of angiogenesis and vessel remodeling (31).

The Tie2, Ang-1 or Ang-2 knockout mice die later in embryogenesis (E9.5~E10.5). The Tie2, Ang-1 or Ang-2 null phenotype showed the vessels are immature, lacking branching networks and proper organization into large and small vessels. There was an absence of angiogenesis in neuroectoderm and in other organ (kidney) (32).

Addition of Ang 1 to VEGF (Ang1+VEGF) produced an increase in macroscopically evident perfusion of the corneal neovasculature without affecting macroscopic measurements of length or circumferential neovascularity. In contrast, pellets containing Ang2+VEGF promoted significantly longer and more circumferential neovascularity than VEGF alone or Ang1+VEGF (33). In particular, these results indicate that angiopoietins may potentiate the effects of other angiogenic cytokines. Moreover, these findings provide in vivo evidence that Ang1 promotes vascular network maturation, whereas Ang2 works to initiate neovascularization.

The complete sequence of Ang-1 and Ang-2 mRNA has been reported in the
GenBank (GenBank/EMBL Accession No. U83508/D13628, and GenBank/ EMBL, Accession No AF00432), and the localization of Ang-1 and Ang-2 gene has also been reported in literature(34). The complete sequence make the detection of Ang-1 and Ang-2 mRNA possible by the polymerase chain reaction.

The role of Ang-1 and Ang-2 on tumor angiogenesis is still unclear, and rarely been investigated. In the previous studies, we investigated the relationship of angiogenic factor (VEGF) and angiogenesis inhibitor (TSP-1) expression to the angiogenesis in non-small cell lung cancer. In this project, we will evaluate the Ang-1 and Ang-2 expression in lung cancer specimens by immunohistochemical staining and by quantitative reverse transcription polymerase chain reaction (RT-PCR). The Ang-1 and Ang-2 expression will be also correlated to the microvessel counts in the lung cancer specimen.

三、研究報告應含的內容

The CT value of Ang-1 mRNA expression in tumor tissue is 33.15 ± 1.96, and in normal tissue is 31.09 ± 2.34. The CT value of Ang-2 mRNA expression in tumor is 31.73 ± 2.02, and in normal tissue is 31.75 ± 1.96.

The ratio of Ang-1 to Ang-2 mRNA expression in tumor (ΔCT = [CT_{Ang-1} - CT_{Ang-2}]) was $2^{-1.47 ± 1.98}$.

The mean survival of these patients was 26.6 ± 12.5 months. When we compared the ratio of Ang-1 mRNA to Ang-2 mRNA expression in tumor with the patient survival, we found that patients with high tumor Ang-1/ang-2 mRNA ratio had longer survival (36 ± 2.54 months, 95% C.I.=31.02~40.98 mo) than patients with low tumor Ang-1/Ang-2 ratio (survival=18.0±2.35 Mo, 95%C.I. =13.4~ 22.6 mo)(p<0.05, log-rank test).

四、參考文獻


