計畫編號：BM01-00
計畫名稱：微核醣核酸與癌症進展
計畫主持人：楊泮池/郭明良
計畫摘要(中)：

微核醣核酸 (miRNAs) 是內生性的小片段 (≈22-nt) RNA，它可以抑制特定序列基因之表現，此作用係透過認識某特定序列互補之 mRNA，而導致轉譯作用之抑制或使 mRNA 在細胞內特定部位分解而達成。在動物中，大部分的微核醣核酸標的基因都具有與微核醣核酸不完全相同的序列。微核醣核酸已經被證實在諸如發育過程、分化、癌症形成、病毒感染與免疫反應等生物功能與基因表現上扮演重要的調控角色，也有越來越多的證據顯示 miRNA 的表現在人類癌症中出現異常。某些特定微核醣核酸的過度表現或低表現，可能與某些特定腫瘤有關。微核醣核酸高度表現，可以導致腫瘤抑制基因的表現下降；反之，若微核醣核酸表現低時，也可能造成某些致癌基因表現增加。最近，miRNA let-7 被發現在肺癌中表現下降，而且 let-7 可以抑制 RAS 的轉譯作用，然而對於特定微核醣核酸在癌症的機制，則尚未闡明。在本研究中，我們企圖探討微核醣核酸在癌症進展中所扮演的角色，特別著重於有關數種台灣重要癌症的轉移、血管新生與細胞存活等相關機制之研究。此外，我們也將建立數個研究平台，包括: 癌症初始細胞之分離與純化、斑馬魚模型之建立、轉殖鼠、生物資訊方法。我們的研究策略包括: (1)運用微陣列分析微核醣核酸基因型與肺癌、乳癌、血癌等重要癌症進展之間的關係；(2) 運用微核醣核酸啟動子庫來研究特定微核醣核酸之基因上調控；(3) 研究微核醣核酸調控血管新生之機轉；(4) 運用血管新生轉換動物模型來研究與血管新生有關的微核醣核酸；(5) 運用基因轉殖動物模型來研究微核醣核酸的功能；(6) 分離並純化癌症初始細胞，並研究其在癌症進展中的角色；(7) 建立斑馬魚模型來研究微核醣核酸的功能；(8) 運用基因轉殖鼠來研究微核醣核酸的功能；(9) 建立分析微核醣核酸基因型表現的生物資訊方法，並運用生物資訊分析平台來研究相關之微陣列結果。

計畫摘要(英)：

MicroRNAs (miRNAs) are endogenous ≈ 22-nt noncoding small RNAs, which negatively regulate gene expression in a sequence-specific

manner. They regulate key aspects of development and physiology in animals and plants. These regulatory RNAs act as guides of effector complexes to recognize specific mRNA sequences based on sequence complementarities, resulting in translational repression or site-specific cleavage. In animals, most miRNA targets are show almost imperfect complementarities with the miRNAs. These evidences indicate that miRNA is a critical regulator for gene expression and biological functions such as development, differentiation, carcinogenesis, virus infection, and immune response. Increasing evidence shows that miRNA gene expression is deregulated in human cancers. Specific over- or under-expression of miRNA has been shown to correlate with particular tumor types. miRNA overexpression could result in down-regulation of tumor suppressor genes, whereas their underexpression could lead to oncogene up-regulation. For example, miRNA let-7 is down-regulated in lung cancer, and has been shown to suppress Ras expression. The mechanisms underlying miRNA gene deregulation in cancer are not well understood. In this research project, we aim to study the roles of miRNAs in cancer progression, focusing on metastasis, angiogenesis, and survival of several important cancers in Taiwan. We will also set up several research platforms including isolation of cancer initiating cells, establishment of a zebrafish model, miRNA knock-out mice, and bioinformatics for studying the functions of miRNAs. Our strategy will be (1) Using the miRNA profiles in different human cancers such as lung cancer, breast cancer, and leukemia to investigate the relationship of miRNA signature and cancer progression; (2) Using a miRNA promoter library to study the epigenetic regulation of selected miRNAs; (3) Investigate miRNA-targeted HIF-1α expression and its regulation of angiogenic activity; (4) Using an angiogenic switch animal model to study miRNA-expression profiles related to angiogenesis; (5) Using transgenic or knockout mouse models to study the function of identified miRNAs; (6) Isolation and characterization of cancer initiating cells, studying their roles in cancer progression and also the miRNA profiling in these cancer initiating cells; (7) establish zebrafish models to study the function of miRNAs; (8) Using conditional knockout mice to study the

functions of miRNAs; (9) Establishment of bioinformatical analysis platform for miRNA profiling, and also using the bioinformatical analysis platform for microarray and proteomic array studies of the downstream genes and proteins regulated by miRNAs.

計畫編號：BM01-01
計畫名稱：微核醣核酸在肺癌轉移的角色
計畫主持人：楊泮池/俞松良
計畫摘要(中)：

肺癌，尤其是非小細胞肺癌，為當今世上最常見的致死癌症之一，而癌轉移則是導致癌症病患死亡的主因。微核醣核酸(microRNA)是新發現的一種不會轉譯成蛋白質的小分子核醣核酸，它可扮演內源性的核醣核酸干擾(RNA interference)，微核醣核酸可透過轉錄後調控機制，調控其標的基因的表現，並可能具有腫瘤抑制基因或致癌基因的功能，微核醣核酸的異常表現在影響癌症轉移能力與預後上或許扮演著重要的角色。我們先前初步分析肺癌病患的 157 個微核醣核酸表現，建立一個 5 個微核醣核酸組成的印記可以作為預測肺癌病患預後與轉移的生物標記。在此計畫中，我們將使用兩種策略來尋找癌症轉移相關微核醣核酸。首先，我們大規模的測量 25 株肺癌細胞株(包括 9 株來自美國國家癌症中心 NCI-60 的肺癌細胞株和 9 株來自 ATCC 的肺癌細胞株及 7 株由台灣肺癌病患建立的細胞株)微核醣核酸的表現，針對每一個微核醣核酸挑選出表現量最低的細胞株，再依據生物資訊學預測微核醣核酸標的基因的可能功能與先前和臨床轉移相關之微核醣核酸的優先順序，將個別的微核醣核酸轉染至相對表現量低的細胞中，以體外細胞侵入性試驗評估這些微核醣核酸對肺癌細胞侵襲性的影響。接下來將這些對肺癌細胞侵襲性有影響的微核醣核酸選殖至表現載體並送入細胞表現，利用二次元差異膠體電泳(two-dimensional difference gel electrophoresis)/質譜儀、西方墨點法、報導基因分析及網路上公開之生物資訊工具，以鑑定這些與癌症轉移與侵襲相關之微核醣核酸的標的基因為何。其次，針對某些癌轉移相關基因(如 HLJ1、caveolin1 等)，這些基因的表現量先前已經證實會影響肺癌細胞的侵襲能力，同時，與台灣肺癌病患的臨床預後有關，我們將以報導基因試驗(reporter assay)篩選微核醣核酸基因庫中對這

些轉移相關基因有轉錄後抑制作用的微核醣核酸。此外，我們將嘗試結合微核醣核酸、與其標的基因建立肺癌預後預測模式。此外，亦將建立以腺病毒為基礎的基因治療(adenovirus-based gene therapy)動物模式，以評估這些微核醣核酸是否具有發展成基因療法的潛力。經由這些努力，我們預期可發現會影響肺癌轉移的重要微核醣核酸及其標的基因，這些發現除了可被利用來當作預後診斷的標記分子外，在未來也可做為治療的標的。

計畫摘要(英)：

Lung cancer, predominantly non-small-cell lung cancer (NSCLC), is the most common cause of cancer deaths worldwide, and metastasis is the major cause leading to mortality for cancer patients. MicroRNAs are a new class of small non-protein-coding RNAs that can act as endogenous RNA interference. MicroRNAs can post-transcriptionally regulate the expression of their target genes and function as tumor suppressors or oncogenes. The aberration in microRNA expression may play a critical role in progression and metastastic competence of cancers. We have previously identified that a five-microRNA signature based on 157 microRNA profiles of NSCLC patients can be served as a biomarker to predict outcome and metastasis of lung cancer patients. In this project, we use two strategies to identify invasion and metastasis-related microRNAs. First, we globally measure the expression of microRNAs in an array of lung cancer cell lines (containing 9 cell lines from NCI-60 anti-drug cell lines, 9 from ATCC and 7 cell lines established from Taiwanese lung cancer patients) to identify which cell produces the minimal amount of individual microRNA. In accordance with the priorities of clinical relevance and bioinformatics analyses the microRNA will be individually introduced into the suitable cell and their effects on metastasis will be functionally analyzed by an invasion assay in vitro. Second, certain metastasis-related genes (such as HLJ1, caveolin1, and others) whose regulatory ability in metastasis and correlation of clinical outcome had been proved in Taiwanese patients will be candidates to screen the microRNAs with post-transcriptional suppressive activity by reporter

assays. Subsequently, the target proteins of these microRNA candidates will be identified by two-dimensional difference gel electrophoresis/mass spectrometry (2D-DIGE/MALDI-TOF) and bioinformatics tools, and further validated by Western blotting and luciferase reporter assays. Finally, the animal model of adenovirus-based gene therapy will be established and employed to evaluate the therapeutic potential of these identified microRNAs on lung cancer. Through our efforts, we anticipate that certain putative metastasis-related microRNAs and their target genes will be identified, and these microRNAs and targets might be employed as prognostic markers, as well as serve as therapeutic targets in the future.

計畫編號：BM01-02
計畫名稱：微核醣核酸在癌症血管新生的角色
計畫主持人：郭明良/俞松良/朱家瑜
計畫摘要(中)：
計畫摘要(英)：

Angiogenesis, the formation of new blood vessels, is a highly coordinated process and is vital for tumor growth and metastasis. So far, there are hundreds of proteins have been identified as pro- or anti-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and so on. Hypoxia-inducible factor (HIF)-1 α is a transcription factor that regulates the supply of blood to tissues through its effects on VEGF expression. HIF-1 α activity in cancer cells could be induced by hypoxia or by hypoxia-independent mechanism such as growth factor stimulation. These oxygen-independent mechanisms are not clearly understood, but they are thought to involve growth factors, cytokines, and other signaling molecules that stimulate synthesis of HIF-1α or decrease its degradation. In addition to that, human tumors commonly harbor mutations in the RAS and MYC proto-oncogenes. These genetic lesions are also potentially pro-angiogenic, as they could activate the production of VEGF.

microRNAs (miRNAs), small noncoding RNAs of ~22 nucleotides,

belong to a novel class of gene regulatory molecules found in plants and animals that negatively control gene expression by binding to complementary sequences on target messenger RNAs (mRNAs). Until now, ~400 of miRNAs have been found in metazoan and some of them were functional validated. Most of these pieces of evidence indicate that miRNA is the critical regulator for gene expression and biological functions such as development, differentiation, carcinogenesis, virus infection, and immune response. However, the role of miRNAs in regulating angiogenic activity of cancer cells is still unclear.

Therefore, in the three-year project, we would like to explore the possible miRNAs that regulate the angiogenic activity of cancer cells and the mechanisms underlying miRNA-targeted regulation of angiogenesis-related genes. To address the above issues, we firstly will identify which miRNAs can specifically regulate the expression of HIF-1

and Ras/Myc, because the importance of both genes in tumor angiogenesis has already been proven. miRNAs which targets HIF-1 or Ras should affect tumor angiogenic activity. In addition, we will establish some animal modes, including angionesis switch model (as described in specific aim 3) to globally screen the human miRNAs. We believe that the three-year project would provide significant results in understanding the roles of miRNAs in tumor angiogenesis and also the progression of cancers.

計畫編號：BM01-05
計畫名稱：微核醣核酸在血癌的角色
計畫主持人：林淑華/周獻堂
計畫摘要(中)：

微核醣核酸(microRNA,miRNA)主控細胞內基因之表現，繼而影響生物生理及分化(1, 2)。已有研究顯示 miRNA 調控造血系統的分裂分化(3)，因此 miRNA 的變異導致血癌的生成是合理的推論，且有證據指出 miRNA 表現量變化在新發的與治療後復發的白血病間具明顯差異(4)，故研究血癌中 miRNA 表現量應能闡釋致病機轉與建立疾病的診斷治療策略。基於此，本計畫目標是研究 miRNA 變化與血癌的致癌機轉相關性，以及研發以 miRNA 作為血癌治療的標靶。

針對上述目標，主持人結合本院醫師與教師的研究團隊，已完成

近 200 例白血病的基因變異與藥物基因體學(投稿中，詳見 Appendix)，也已利用定量 RT-PCR (反轉錄¬-聚合酶連鎖反應)方法發現 4 種 miRNA 中有三種在兒童急性白血病的表現量具統計意義的變化，值得發表，最近更進一步利用本院卓越中心基因微陣列核心設施的尖端科技作 365 種 miRNA 的分析，初步成果顯示臺灣的兒童白血病具有特定的 miRNA 變化影響到病人的預後，因此本團隊希望完成下列主題：

1. 分析血癌中 miRNA 的表現圖譜，希望據此選擇治療的方針。
2. 研究調控 miRNA 與下游受其調控的基因，藉以釐清 miRNA 在血癌發展及治療中的角色，並了解血癌的致病機轉。
3. 利用基因剔除與轉殖技術製造過量或低量表現血癌特異的 miRNA 的血癌小鼠模型以研究致病機轉並提供治療血癌的動物模型。

計畫摘要(英)：

MicroRNA (miRNA) plays important roles in cell proliferation and differentiation(1, 2). Several miRNA has bee suggested to regulate hematopoiesis(3). Thus, aberrant miRNA expression may lead to blood cell cancer (leukemia/lymphoma). Indeed, this has been proven in some leukemia/lymphoma before and after treatment(4). Conceivably, exploring miRNA expression profiles in leukemia/lymphoma and their impacts on cancer development and drug response is of particular interest not only for the purpose of understanding disease mechanism but also with a vision that miRNA might be a candidate for pharmaceutical targets and for prognostic marker.

The Principal Investigator has organized on campus a team of physicians and research scientists with hematology background and expertise in transgenic/knockout mice. The team has collected nearly 200 leukemia cases and studied their pharmacogenomics and treatment outcome (manuscript submitted, see Appendix for detail). We have also used quantitative RT-PCR (reverse transcription-polyerase chain reaction) in a pilot study and identified 3 of 4 miRNAs) being differentially up- and/or down-regulated in acute childhood leukemia (manuscript in preparation). In collaboration with the Microarray Core (under Dr. Yang and Dr. Yu, the P.I. of subproject 1’s authority) on campus, we have

further profiled 365 miRNA on our leukemic samples and have identified important miRNA expression signatures for childhood leukemia with and without remission. With these progresses we propose the following aims.

1. To dissect the miRNA expression profiles in leukemia aiming at predicting treatment outcomes. The long term goal is to set up a miRNA signature for disease follow-up.
2. To investigate the upstream regulator and downstream target of miRNAs responsible for disease progression and clinical outcome. The long term goal is to understand the pathogenic mechanisms where miRNAs are responsible for childhood leukemia.
3. To generate transgenic and gene-targeted mouse models with over expression and elimination, respectively, of the miRNA associated with childhood leukemia. The long term goal is to generate a mouse model for research on translational medicine.

計畫編號：BM01-06

計畫名稱：微核醣核酸調控機制與其作用標的之預測

計畫主持人：莊曜宇/賴亮全

計畫摘要(中)：

本計劃的主要目的是了解 microRNA 在癌症發展中所扮演的角色，其它子計劃會依此主軸做一系列的研究並產生許多資料。故我們必須建立一資料庫來整理這些資料、並提供生物資訊的工具來分析這些資料，以助尋找 microRNA 標的基因。故此，本子計劃的目的是建立使用者容易操作的網路平台，以利 microRNA 研究的進展。

在先前建立之「國立台灣大學微陣列分析平台及系統」(NTUMAPs)的架構下，我們將添加分析檢測 microRNA 的線上工具，以幫助其它子計劃尋找新的 microRNA。將採用分析的方式有二：第一，使用機器學習計算法來尋找新的 microRNA。我們先用已知的 microRNA 序列來訓練程式，再從可能的候選 microRNA 中、尋找那些 microRNA 的序列有演化上保留的髮夾序列、最低的自由能、及最相似已知的 microRNA，來尋找新的 microRNA。其次，我們也可比較從 microRNA 檢測中所得的候選 microRNA 序列，與從 mRNA 表

現降低的基因中、其 3’ UTR 所找到的序列；藉由尋找相似的序列，來找新的 microRNA。

在找到新的 microRNA 後，我們會更進一步提供預測 microRNA 之標的基因的服務。雖然現有一些預測 microRNA 標的基因的程式，但這些演算法提供太多錯誤的預測；故需花許多時間去驗證，也使得這預測資訊的可用性不高。故此，我們將致力於發展一減少錯誤預測的方法，來提高預測 microRNA 標的基因之準確率。我們提出的策略是結合 Hidden Markov Model 預測最大相似度方法，Bayesian 資料分析計算法，演化上功能保留程度，及從相似表現基因的 3’ UTR 中尋找共同序列等方法。另外，我們也會用 Hidden Markov Model 發展模擬模型，以預測不同 pre-microRNA 的突變與結構對其標的基因的影響。為了驗證我們的方法，我們也會用癌症細胞，做一些 microRNA 及基因表現的實驗。

當發展出較精確的預測方式後，我們會建立一整合性平台來結合 microRNA 與 NTUMAPs 現有的其他基因體資料。我們的資料庫將不只提供 microRNA 及其標的基因之詳細資訊，也會整合基因表現，DNA 數目，及蛋白體的資料。這整合性的資料會助於我們了解 microRNA 調控基因表現對癌症發展的影響。總而言之，藉由整合資訊與實驗的資料，我們擁有前所未有的機會、來系統地研究 microRNA 調控基因表現、對癌症發展的影響。

計畫摘要(英)：

According to the main goal of this project to understand the role microRNA plays in cancer progression, other sub-projects will make a series of biological studies to search the candidate microRNAs enriched in metastasis and angiogenesis in cancer cells, and will generate massive data. Then, it is necessary to develop a database to organize these data and some bioinformatic tools to analyze these data as well as provide guidance to pursuit microRNA target genes for following functional analyses. Therefore, the purpose of this sub-project is to establish a user-friendly web-based platform to facilitate the microRNA research progress.

Based on the framework of NTUMAPs (National Taiwan University Microarray Analysis Platform & System), we will include an online analysis platform for microarray-based microRNA assays and help other

sub-groups to identify new microRNA genes. First, machine learning algorithms will be used to search new microRNAs. After training the program with known microRNA sequences, we can identify the new microRNAs by searching which enriched nucleotides from microRNA assays (the microRNA candidates) contain conserved hairpin sequences, minimum free energy of hairpin structure, and are most similar to the training data. In addition, we may identify new microRNAs by comparing the candidate microRNA genes from the microRNA assays with the de novo sequence motifs which can be found from the 3’ UTR of genes with lower levels of mRNA, and searching the best correlated sequences.

Next, after identifying new microRNAs, we will further provide the prediction of microRNA target genes. Although there are some programs available to predict the microRNA target genes, the main problem of current algorithms is providing too many false positives, which needs a lot of time to validate possible target genes and makes the prediction information less useful. Therefore, we will devote ourselves to define some criteria in order to decrease the false positives in predicting the microRNA target genes. The possible criteria to eliminate false positives include filtering genes on tissue- or developmental stage-specific expression, genes constraining to similar functional groups, and genes containing multiple target sites or conserved target sites, etc. Also, we will develop a simulation model by using the Hidden Markov Model (HMM) to estimate the effects of different mutations and structures of pre-microRNA on its target sites. In order to validate our algorithm, we will generate some real data by using human cancer cells for profiling microRNA and gene expression respectively.

After developing more accurate predicting algorithm, we will set up a platform that combines microRNA data with their related microarray data in the NTUMAPs. Our database will not only provide detailed information for microRNA profiling and the predicted microRNA target genes but also integrate gene expression, DNA copy number, and other proteomic data. By considering microRNAs and their targeted gene/protein expression profiles at same time, it will greatly facilitate our

understanding on gene regulation of cancer progression, which is mediated by microRNAs. To sum up, by integrating the genome-wide computational and experimental data, we have the unprecedented opportunity to study function of gene regulatory control mediated by microRNAs at a system-wide level in cancer progression.