行政院國家科學委員會專題研究計畫 成果報告

microRNAs 標的遺傳序列之甲基化形式和功能表現在婦女乳癌進程之角色探討

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一、中文摘要

乳癌為台灣女性第二常見的腫瘤，其發生率和致死率近十年內增加，且有年輕化之趨勢。基於癌細胞發生腋下淋巴或者是遠端組織器官的腫瘤轉移，往往對生命產生嚴重的威脅性。對婦女乳癌而言，探討乳癌癌化進程乃至於發生淋巴轉移的機轉，便有其特殊意義的急迫性。本研究計畫中，我們嘗試在腫瘤細胞的發展過程中，以定量RT-PCR技術針對特定標的基因的表現量，探討與乳癌腋下淋巴轉移發生的關聯。透過雷射顯微擷取(laser capture microdissection)將其乳癌組織和乳部相鄰之正常組織檢體加以分離後，進行qRT-PCR實驗分析，結果發現在乳癌預後相關的miRMA標記中，(一) *miR-21* 在癌組織表現增加和腋下淋巴轉移(lymph node metastasis)有關；(二)高度表現*miR-221* 和乳癌分期及腋下淋巴轉移相關(P<0.05)。另外，*miR-200*低度表現和臨床分期有關。(三)然而，在ER和PR表現的乳癌組織中，*miR-200*的表現在乳癌臨床預後的評估上呈現正相關。(四)乳癌分期和腋下淋巴轉移會明顯受到*miR-21*和*miR-221*協同表現的影響。總結而言，針對多重miRNA序列的複合性表現程度的探討，其結果將有利於對台灣地區婦女乳癌發展進程提供完整重要的研究訊息，更可以做為國人婦女乳癌預後的評估指標。

關鍵字：乳癌， miRNA，雷射顯微擷取，動情素受體蛋白，遺傳標記

二、英文摘要

The intrinsic nature of gene dysregulations resulting in cell oncogenesis remains an issue of extraordinary complexity. MicroRNAs (miRNAs) represent a novel class of short non-coding RNA molecules that negatively regulate expressions of oncogenes and TSGs involved in cancer pathogenesis. To address the mechanism of epigenetic control by miRNA at various stages of breast cancer, expression patterns of miRNAs, including *has-miR-21*, *-200c*, and *-221* from laser capture microdissection (LCM) collected paired cancer and normal tissues of breast. These were examined using quantitative real-time polymerase chain reaction (qRT-PCR) to define their tumorigenic contribution. Expression level of miRNAs in each cancer patient was defined as the ratio of the amount of miRNAs between tumor and normal tissues with the internal positive control of *RNU6B.* We found that (i) *miR-21* was overexpressed in breast tumor relative to matched normal control, manifested a significant correlation with lymph node metastasis (P<0.05). (ii) Increased expression of *miR-221* was present in tumor tissues, showing a significant association with advanced clinical stage and lymph node metastasis (P<0.05). *miR-200c* was observed to be down-regulated in poorly-differentiated tumors. (iii) However, increased expression of *miR-200c* correlation was found in cases categorized as positivity for estrogen receptor or progesterone receptor. (iv) A joint effect of *miR-21* and *miR-221* overexpreesion was shown in advanced tumor stage and nodal metastasis of breast cancer (P<0.05). In conclusion, epigenetic aberration of miRNAs can predict early events in the multistage progression of cancer disease. Overexpression of *miR-21* and *miR-221* favors the induction of the malignant phenotype, and consequently, may be used as molecular prognostic markers for disease progression of breast cancer in the future.

Key words: Breast cancer, *miRNA*, Laser captured microdissection, qRT-PCR, marker

報告內容

一、計畫緣由與目的

Tumorigenesis is a multistep process resulting from a series of genomic alterations which lead to the progressive disordering of the normal mechanisms controlling growth, death, and differentiation of the cell [Fearon and Vogelstein, 1990]. To account for the high frequency of genomic alterations required for tumor progression, it has been suggested that the genomes of cancer cells are unstable and that mutators cause these genomic instabilities (the "*mutator phenotype*" theory) [Leob, 1998]. Theoretically speaking, for tumor cells progression, the pre-malignant lesions are caused either by genetic alteration and/or by epigenetic mutations. Accumulations of these genetic alterations occur in a few of the pre-malignant cells, converting into malignant ones of clonal origin and become a phenotype primary tumor [1]. Stepwise progression of human tumor cell pre-malignancy had been detected in diverse organs prior to the appearance of fully malignant invasive tumors. In advance, after the early stage of primary tumor expansion, the new clones with invasiveness and metastasis appear as a result of further accumulation of genetic alterations in the cells [2, 3]. Based on our observations of genome-wide study within laser capture microdissected cells (LCM-cells) for detecting loss-of-heterozygosity, we demonstrated that the extents of oncogenes and TSGs in breast tumors significantly altered as tumors progressed to poorer grades or later stages, leading to essential evidences to support the importance of cellular responses during breast tumorigenesis [4]. Therefore, the "mutator phenotype" hypothesis suggests that, to account for the high frequency of genomic alterations (i.e. genomic instability) for development of tumor cells [5].

Alterations of protein-coding oncogenes and tumor suppressor genes (TSGs) have been thought to be the causes of tumorigenesis and driving for the metastatic process. Recent investigations of oligonucleotides that non-coding RNA transcripts with no significant coding can lead to silencing of the target genes through the RNA interference pathway which was named microRNAs (miRNAs) [6, 7]. More importantly, recent evidences indicated that some miRNAs may effect on either oncogenes or TSGs in contributing to cell tumorigenesis [8]. Thus, the analyses of expression profiles can yield characteristic of miRNA signatures in human cancers. The tumor-suppressor phosphatase with tensin homology (*PTEN*) is the most important negative regulator of the cell-survival signaling pathway that is initiated by PI3K. In the face of DNA strand break, PTEN is being an inhibitor of PI3K pathway to result in the sequestration of cell cycle checkpoints, being a collaborator and an effector of $p27^{kip1}$ tumor suppressor to cause G1/S cell arrest, being a transcriptional regulator of DNA damage repair proteins, and being a modulator of constitutively regulating Wnt/β-catenin-dependent signaling. To understand the mechanism linked to PTEN-regulated expression in contribution to tumor progression in breast cancer, a multigenic investigation of miRNAs is to be performed using paired cancer and adjacent normal tissues collected via laser capture microdissection (LCM) technique. Those laser-captured cells from both tumor- and non-tumor cells of each patients were subjected to real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) for examination the differential expression levels of miRNAs in *hsa-mir-21* (*PTEN*; *Pdcd4*), *hsa-mir-221* ($p27^{kip}$) and *hsa-mir-200c*

(*E-cadherin*). This study therefore speculates targeted miRNAs, *miR-21*, *miR-200c* and *miR-221*, acting as suitable molecular markers of prognostic evaluation in breast cancer.

二、研究方法

Questionnaire

An experienced research nurse was assigned to administer a structured questionnaire to each breast cancer patients. The information collected included age at diagnosis, family history of breast cancer (first-degree relatives), history of breast biopsy, history of breast screening, age at menarche and/or menopause, parity, age at FFTP, number of pregnancies, history of breast feeding, use of oral contraceptives, HRT, history of alcohol consumption and cigarette smoking, ethnic background, residence area, family income, and education level. The BMI and menopausal status were also recorded.

Laser capture microdissection (*LCM*)

To ensure that tissue samples assayed consisted of >90% tumor cells, LCM was performed on routinely immunostained slides using a PixCell laser capture microscope (Arcturus Engineering, MountainView, CA) as described previously [Emmert-Buck, et al., 1996] with minor modification. Briefly, the stained, dehydrated tissue section was overlaid with a thermoplastic film mounted on an optically transparent cap. The visually selected areas (tumor cells) were bound to the membrane by short, low-energy laser pulses, resulting in focal melting of the polymer. The LCM captured cells were immersed in 50-100 μl of digestion buffer, containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 400 μg/ml proteinase K, and 1% Tween 20, and digested at 55°C overnight. After digestion, the enzyme was heat inactivated (95°C for 10 min), and the extractwas used directly for RNA isolation.

RNA preparation and detection of the targeted gene expressions by qRT-PCR

Total RNA was extracted from tumors and normal breast tissues of individual cases using an RNA extraction kit (RNeasy) (QIAGEN, Valencia, CA, USA). Stepwise, the RNA (1 μg in a volume of 5 μl) was reverse-transcribed for 70 min at 42°C using 5 units of Superscript II reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) and 10 mM random primers of oligo(dT) 15 primer (Promega, Madison, WI) in a reaction volume of 20 μl. cDNA concentrations were determined by spectrophotometry. Substantially for qRT-PCR reaction, 25 ng of the total RNA-reverse transcribed cDNA product was subjected in totally 25 μl of Universal PCR Master Mix (Applied Biosystems), 900 nM forward primer, 900 nM reverse primer, 200 nM probe and nuclease-free water were added to a final volume of 50 μl. Amplification and detection steps were performed with the ABI Prism 7700 sequence detection system (Applied Biosystems). RNU6B gene was used as the internal positive control in each qRT-PCR batch. The differential expression level of the relevant gene in cancer patient defines as the ratio when the tumor tissue and surrounding non-cancer tissue of breast were compared in each case. The relative amount of each target gene mRNA was calculated as the average 2^{-ddCt} where $ddCt = Ct_{taregt} - Ct_{RNU6B}$.

Data Analysis

To test our hypothesis, we examined whether there was a correlation between the expression levels of *miR-21, miR-200c* and *miR-221* genes in cancer tissue and the tumor pathological features, including tumor size, stage, grade, and LNM. Quantitation of the gene transcripts was determined according to formal definitions. Expression level of the individual genes was estimated as the ratio of two measurements (i.e., the target gene/RNU6B ratio in the cancer tissue vs. this ratio in the corresponding normal tissues). The significance of the association was assessed by using the two-sided chi-square test, Fisher's exact test, and the Mann-Whitney U-test between groups where appropriate. A *P*-value < 0.05 indicates statistical significance.

三、結果與討論

To evaluate the role of differential expressions of these miRNA markers in association with breast cancer development, 65 patients histologically proven IDC of the breast underwent curative mastectomy at department of surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan. The clinico-pathological characteristics of studied breast cancer patients were summarized in Table 1. Among them, 73.8% (48 out of 65) tissues were clinically staged as earlier tumor disease (Stage I and II) and 26.2% were tumor of poorer differentiation (Stage III and IV). Negative examination for estrogen receptor was found in 26 patients (41.9%), by contrast, 36 (58.1%) cases were ER-positive at the time of diagnosis, respectively. Case of LNM-positive was examined 53.2% (33 out of 62) of the IDC patients. Additionally, all the patients have been approved by the ethics committee board of the department of surgery, Tri-Service General Hospital, National Defense Medical Center.

The paired primary tumor cells and corresponding adjacent non-tumor on the same patients of breast tissues were carefully separated by laser capture microdissection (Fig. 1). Stepwise, those cDNA transcripts were all subjected into qRT-PCR analysis for examining the targeted genes, including *miR-21*, *miR-200c* and *miR-221,* and RNU-6B gene was used as the internal control in each qRT-PCR batch. By comparing cancerous and tumor-adjacent normal tissues, we found that there was a positive association between the increased *miR-21* and *miR-221* expressions and patients classified as advanced tumors (stage IIb, III, and IV) (Fig. 2). As shown in Table 2, m*iR-21* was overexpressed in breast tumor relative to matched normal control, manifested a significant correlation with lymph node metastasis ($P < 0.05$). Increased expression of *miR-221* was detected in tumor tissues, showing a significant association with advanced clinical stage and lymph node metastasis ($P < 0.05$). On the other hand, *miR-200c* was observed to be down-regulated, but not significant, in association with poorly-differentiated tumors (Table 2).

Prognostication of breast cancer using clinicopathologic variables (such as ER and PR classifications), though useful, remains inconclusive. To consider the prognostic surrogate for breast cancer, we examined expression levels of these three miRNAs in tumor patients who were stratified by hormone receptors. As shown in Table 2, it appears that the mean expression levels of

miR-200c transcripts were significantly higher in ER-positive tumors (3.64±2.00) than that detected in ER-negative tumors (1.28±2.35) ($P = 0.02$). Similarly, increased expression of *miR-200c* was found in tumors of PR-positive (3.56±2.07), which is significantly higher than that of cases of PR-negative (1.37±2.37) ($P = 0.03$). Moreover, in ER-positive breast cancers, an increased mRNA level of the *miR-200c* was found in high LNM-positive tumors ($P = 0.003$). Compared to normal breast tissues, mRNA level of *miR-200c* was higher in PR-positive breast cancer tissues, and such epigenetic changes did correlate to the clinicopathological pathology of tumors with advanced tumor stage and LNM ($P < 0.05$) (Table 3).

To delineate the synergistic effects of the differential expression of the *miR-21, miR-200c* and *miR-221* in association with breast tumor development, all patients were divided into groups on the basis of clinical findings. In this multivariate analysis, by using a dummy variable coding scheme and the β estimate from the regression model [9], the joint effect of increasing the risk associated with metastatic tumors was observed in women carrying a great number of overexpression of the *miR-221 and miR-200c* (aOR = 2.15 95%CI =1.29-3.61). Likewise, the effect of the overexpression of both *miR-221* and *miR-21* levels was associated with a 2.82-fold risk increase in advanced stage tumors, however, this lacks a significant association (Fig. 3).

To the best of our knowledge, this will be the first study to address the issue of an interaction between *PTEN*-associated mechanisms in relation to breast cancer risk by determining the expression pattern of miRNAs. These epigenetic polymorphisms of defining putative TSGs and/or oncogenes would *predispose* carriers to a higher risk of developing cancer. Knowledge of this study were examined the possibility of the tumorigenic phenotype based on the comprehensively evaluate the miRNA-regulated genes participating in tumorigenesis and tumors of different pathological and clinical stages to reflect the sequential steps occurring during tumorigenic progression [10]. In an attempt to elucidate the etiological and phenotypic complexities, the present study proposed a tumor progression model to explore breast cancer tissues by which classifications are carefully being manifested the statuses of tumor stage, grade, ER/PR, lymph node metastasis and disease survival, and thus, miRNA reports of this study can provide the molecular markers for prognostic evaluation in breast cancer.

It has been reported that PTEN acts as a target of miRNA-21. miRNA-21 binds to 3'-untranslated region of PTEN mRNA and induces its degradation via an mTOR/NF-kB-dependent behavior, and thus triggers down-regulation of PTEN mRNA in liver disorders [11]. Direct evidence implicating loss of PTEN activity in causal induction of mammary tumors derives from both germline and conditional knockouts of PTEN in a number of animal model systems [12-14]. PI3K mutations were found to be associated with expression of estrogen and progesterone receptors (ER/PR), lymph node metastasis, and ERBB2 overexpression, and PTEN loss to ER/PR-negative tumors [15, 16]. The effects of heterogeneity within a tumor cell were minimized by ensuring that genetic and phenotypic examinations are being carried out on the same tumor tissues using laser capture microdissection (LCM), allowing a more precise evaluation of specific associations between genetic alteration and pathological manifestation of breast cancer in Taiwan. Our data hereby demonstrated that regulated

expressions of miRNA-related to *PTEN*, $p27^{kip1}$ and *E-cad/β-catenin* signaling [17]may associate with the development of breast cancer. To the best of our knowledge, our study ins the first to establish a methodological framework involved in such multistep process by examining the multiple gene expressions, including *miR-21*, *miR-221*and *miR-200c*, are respectively responsible for regulation of breast tumor cell processing. Induction for cell proliferation through loss of *miR-200c* expression implicated that *β-catenin* releases and accumulates in the cytoplasm, was significant in the initiation of an invasive phenotype.

In an effort to establish a methodological framework for analysis of molecules and mechanisms involved in this complex multistep process, we aimed at developed an experimental system with quantitatively qRT-PCR in detecting these three candidate genes in relation to the evaluation of tumor development. The effects of heterogeneity within a tumor were minimized by ensuring that genetic and phenotypic examinations are being carried out on the same tumor cells by using the method of laser capture microdissection (LCM) which allows a more precise evaluation of specific associations between genetic and pathological manifestation. In conclusion, epigenetic aberration of miRNAs can predict early events in the multistage progression of breast cancer. Overexpression of *miR-21* and *miR-221* favors the induction of the malignant phenotype, and consequently, can be used as molecular prognostic markers for disease progression of breast cancer in the future.

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五、計畫成果自評

本研究根據民國九十一年起至九十六年期間，自三軍總醫院婦女乳癌病患所建立起癌症組織資料庫，以雷射顯微擷取(laser capture microdissection)分離癌組織和相鄰之正常組織後，再以反轉錄聚合酶連鎖反應將cDNA加以進行轉錄。利用定量Real-Time RT-PCR將乳癌患者之乳房病灶及其相鄰正常乳房上皮組織為研究材料、結合臨床問卷調查，探討與淋巴移轉相關miRNA表現程度差異的分子遺傳學研究，以釐清miRNA遺傳標記在婦女乳癌發展過程中所扮演的角色。我們發現*miR-21*和*miR-221*的表現程度與乳癌乳癌分期和腋下淋巴轉移達顯著統計相關。再者，*miR-21*和*miR-200c*的表現會分別協同*miR-221*影響乳癌之淋巴轉移。綜合而言，本研究結果首次探討miRNA的表現對於乳癌臨床預後評估的重要性。藉由對多重基因的變項分析，更精準的以雷射捕獲組織檢體，加以定量基因檢測分析，為國內建立起基因表現程度標記與乳癌發生、分期、腫瘤轉移和預後關聯性研究的資料庫。其研究成果可提供解釋乳癌惡化病程的可能機轉和其相關聯的遺傳標記；更可以進一步以此做為未來發展有效的乳癌治療策略。

六、圖表

Table 1. Clinical features of 65 female patients with IDC breast cancer

Characteristics	N (%)
Age (Mean±S.D.)	49.2±13.4
Tumor size (cm)	
T1	36 (56.4)
T2	22 (33.8)
T3/T4	7 (10.8)
Grade	
I	19 (32.2)
II	24 (40.7)
III	16 (27.1)
Stage	
I	27 (41.5)
II	21 (32.3)
III/IV	17 (26.2)
Node	
Negative	29 (46.8)
Positive	33 (53.2)
ER	
Negative	26 (41.9)
Positive	36 (58.1)
PR	
Negative	24 (43.6)
Positive	31 (56.4)
Erbb2	
Negative	25 (52.1)
1+	4 (8.3)
2+	7 (14.6)
3+	12 (25.0)

Table 2. miRNAs are differentially expressed in histological breast tissue of the IDC patients compared to corresponding normal breast tissues

	miRNA expression level (-ddCt) (Mean±S.D.)								
Characteristics[a]	*miR-200c*	*P**	P_{trend}	*miR-21*	*P**	P_{trend}	*miR-221*	*P**	P_{trend}
Tumor size									
T1+T2	2.43±2.41	0.83		2.45±2.58	0.63		2.31±2.64	0.60	
T3+T4	2.16±3.41			3.11±3.15			3.02±1.70		
Grade			0.60			0.38			0.23
Ⅰ	1.33±2.84			1.15±2.09			1.31±2.44		
Ⅱ	3.08±2.36	0.15		3.18±2.71	0.06		2.51±2.51	0.28	
Ⅲ	2.28±2.36	0.39		2.52±2.60	0.21		2.76±2.67	0.21	
Stage									
I+II	2.60±2.21	0.55		2.41±2.81	0.78		2.10±2.32	0.42	
III+IV	2.13±2.87			2.64±2.35			2.77±2.90		
Node									
Negative	2.47±0.56	0.88		1.59±2.65	0.03		1.71±1.93	0.02	
Positive	2.35±2.54			3.39±2.29			3.31±2.78		
ER									
Negative	1.28±2.35	0.02		2.72±2.70	0.95		2.21±2.68	0.45	
Positive	3.64±2.00			2.67±2.37			2.82±2.32		
PR									
Negative	1.37±2.37	0.04		2.80±2.69	0.80		2.38±2.77	0.71	
Positive	3.56±2.07			2.60±2.37			2.68±2.66		

Erbb2			0.60			0.54		0.83
Negative	2.35±2.77			2.94±2.12			2.41±2.43	
1+	3.05±4.35	0.86		2.89±6.40	0.99		3.40±3.61	0.60
2+/3+	2.69±2.08	0.67		2.44±2.55	0.51		2.58±2.56	0.83

[a] The expression levels for individual miRNA genes were obtained by comparing primary tumor tissue with the tumor-adjacent normal tissue from the same patient with breast cancer. Well differentiation represents stage I and II and poor differentiation represents stage III and IV, respectively. *, $p<0.05$.

Table 3. Association study of differentially expressed miRNA levels of *miR-21*, *miR-200c* and *miR-221* and clinical features stratified by ER, PR, and Erbb2

	miRNA expression level (-*ddCt*) (Mean±S.D.)					
Characteristics	*miR200c*	*P**	*miR-21*	*P**	*miR-221*	*P**
ER-positive						
Stage						
I+II	2.58±2.93	0.83	3.34±1.75	0.38	2.63±2.32	0.64
III+IV	2.49±2.60		4.14±2.40		3.13±2.47	
Lymph node status						
Negative	1.28±1.69	0.003	3.04±1.86	0.15	1.85±1.70	0.05
Positive	4.20±2.09		4.32±2.04		3.90±2.52	
PR-positive						
Stage						
I+II	2.64±2.54	0.92	3.49±2.09	0.83	2.61±2.23	0.84
III+IV	2.52±2.19		2.70±2.22		2.82±2.50	
Lymph node status						
Negative	1.84±2.49	0.09	3.35±2.14	0.60	1.87±1.63	0.06
Positive	3.61±1.86		3.85±2.09		3.76±2.62	
Erbb2-positive						
Stage						
I+II	4.34±2.36	0.02	3.61±2.67	0.73	1.17±2.41	0.02
III+IV	2.75±0.58		1.78±1.77		318±2.14	
Lymph node status						
Negative	0.35±1.41	0.03	2.86±1.63	0.83	2.03±1.59	0.39
Positive	3.80±2.67		2.64±2.55		3.05±3.03	

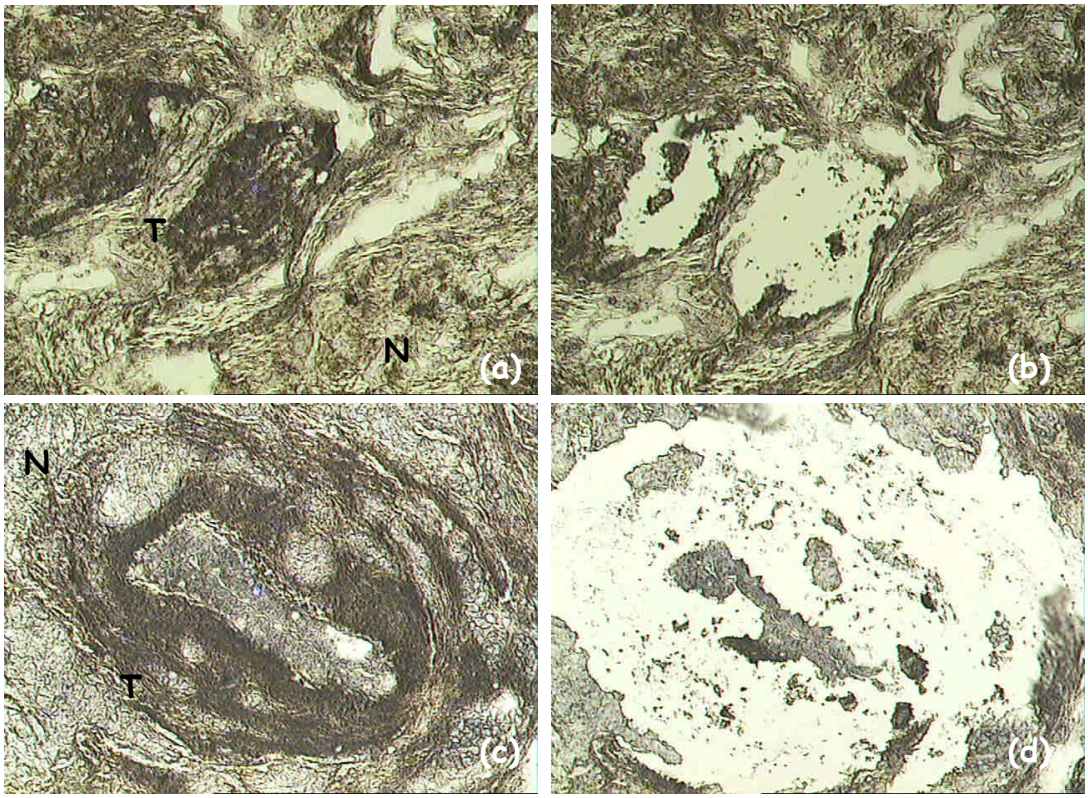
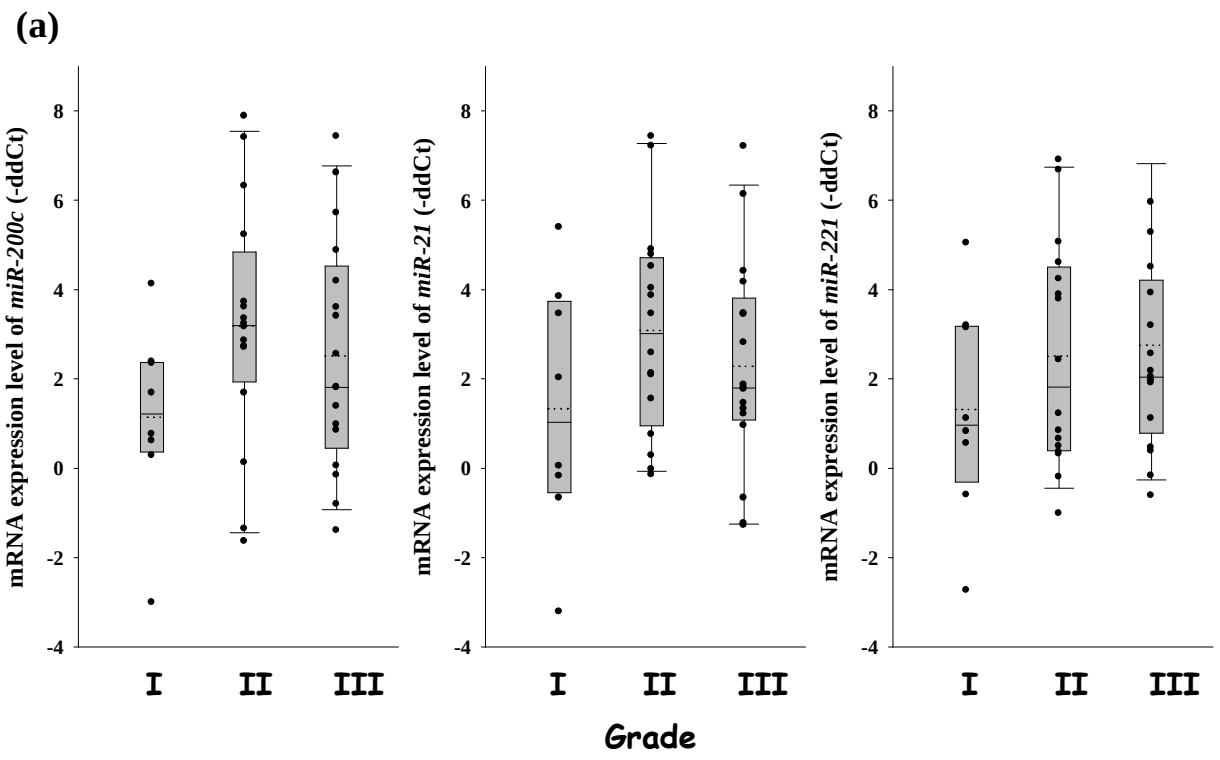
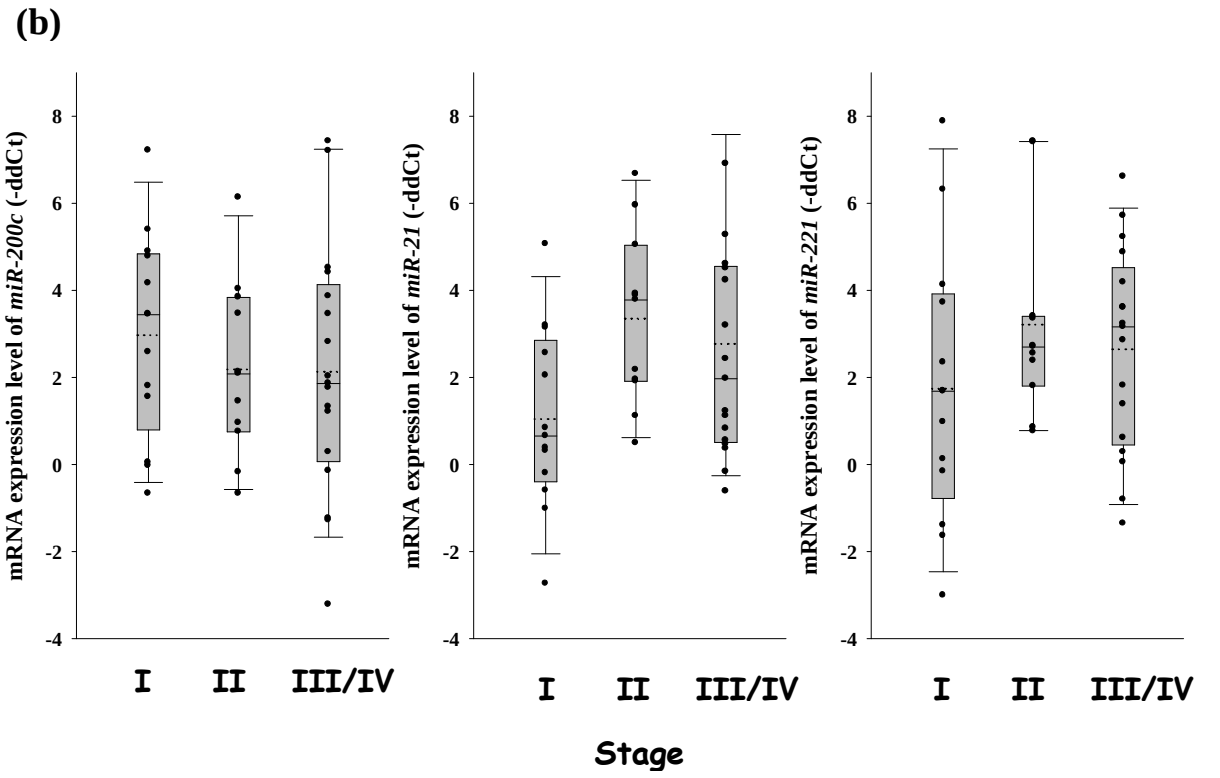


Fig. 1. Isolation of breast tumor cells using laser capture microdissection technique. (a) and (c) are breast cancer tissue resections before LCM treatment. T, tumor and N, tumor-adjacent normal part; (b) and (d) are Post-LCM image of laser capture. Cancer tissues collected from breast cancer patients who were characterized as T1, stage I and negativity of lymphnode metastasis (N0).


(a)
mRNA expression level of miR-200c (-ddCt)
mRNA expression level of miR-21 (-ddCt)
mRNA expression level of miR-221 (-ddCt)
8
6
4
2
0
-2
-4
I
II
III
Grade


(b)
mRNA expression level of miR-200c (-ddCt)
mRNA expression level of miR-21 (-ddCt)
mRNA expression level of miR-221 (-ddCt)
8
6
4
2
0
-2
-4
I
II
III/IV
Stage

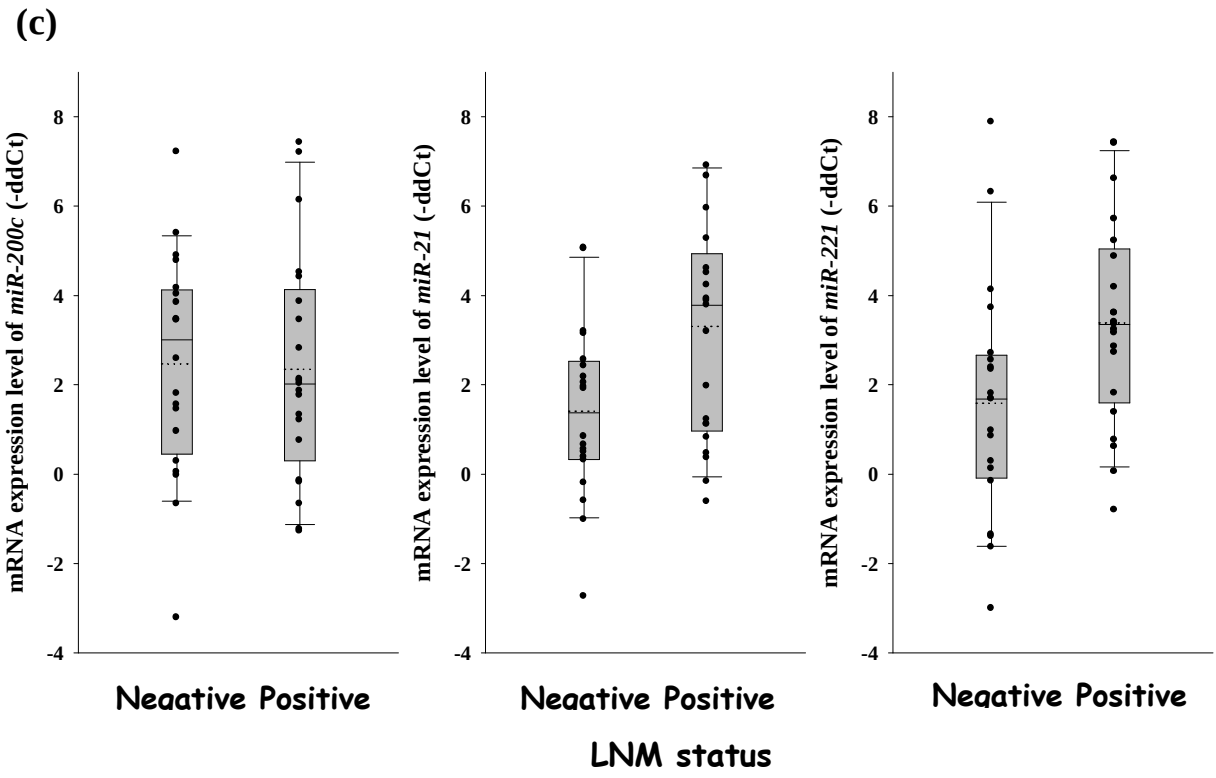
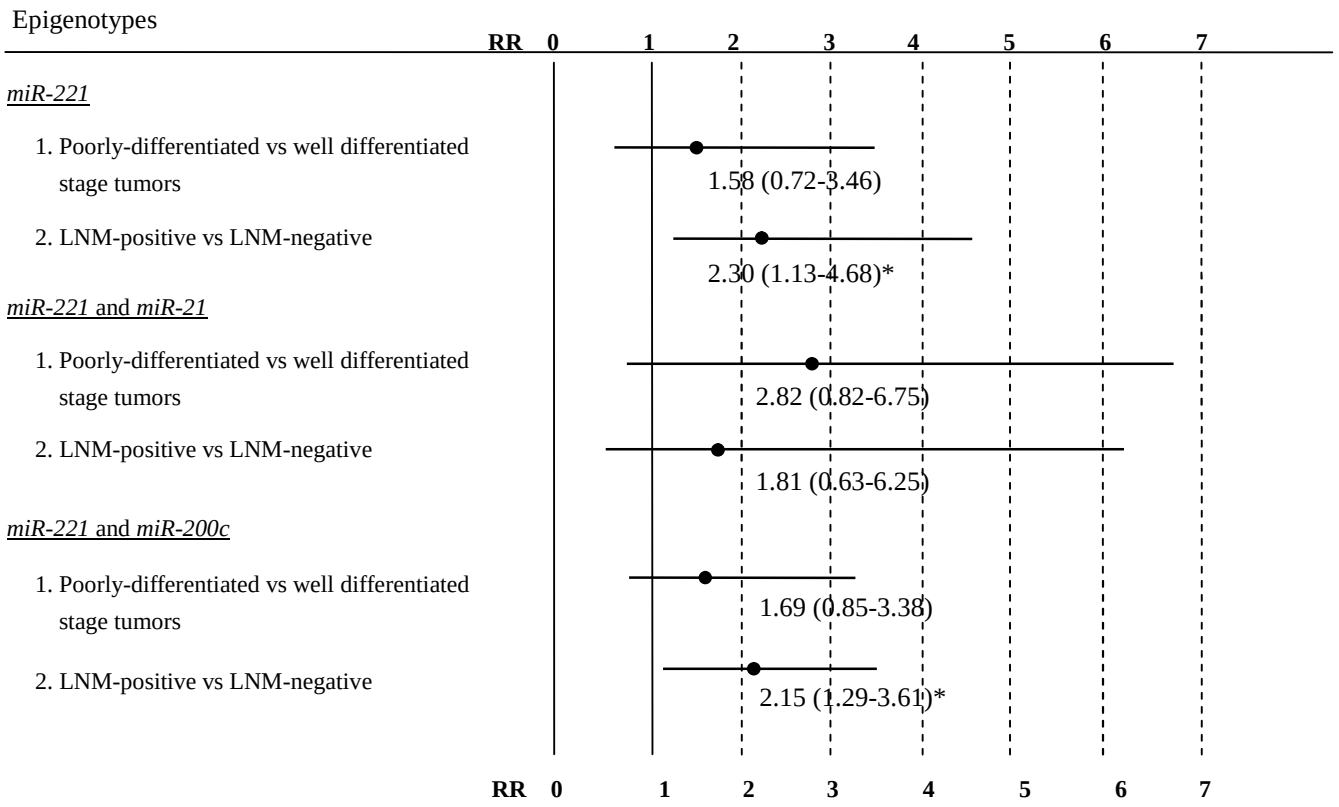


Fig. 2. qRT-PCR analyses of miRNA expression in correlation to the clinical relevance of breast cancer. LCM-treatreated tumor tissues and neighboring non-tumor tissues from IDC patients were subjected to quantitative analysis of *miR200C*, *miR-21*, and *miR-211* levels, and levels of which were associated with IDC among well-known prognostic factors, including grade (a,upper), stage (b, middle), and LNM (c, lower).

Fig. 3. Gene-to-gene interactions between *miR-221*, *miR-21*, and *miR200c* in association with breast cancer



*, $P<0.05$.

出席國際學術會議心得報告

計畫編號	97-2314-B-040-013-
計畫名稱	microRNAs標的遺傳序列之甲基化形式和功能表現在婦女乳癌進程之角色探討
出國人員姓名 服務機關及職稱	鄭鈞文 中山醫學大學生化暨生物科技研究所副教授
會議時間地點	April 16-18, 2009 ; Kyoto, Japan
會議名稱	Kyoto Breast Cancer Consensus Conference, 2009 International Convention
發表論文題目	Clinical implication of miR-21 and miR-221 in relation to progression of human breast cancer

一、參加會議經過

2009 年日本京都乳癌國際會議 (Kyoto Breast Cancer Consensus Conference, 2009 International Convention) 於二千零九年四月十六日至十八日，為期三天，在日本京都國際會議中心召開。本次大會有來自全球各癌症臨床醫學相關學術機構、業界之專業研究人員，參與此次乳癌基礎和臨床專科會議。本次所參與發表的論文摘要於三天的會議中分別以專題研究發表、專題討論及論文海報張貼等方式同時進行。大會進行中，首先針對前哨性腋下淋巴結為乳癌臨床之研究標記，以 indocyanine green fluorescence 探討與乳癌發展進程之關聯。其中，臺大醫學院外科黃俊升醫師受大會邀請以診斷探討 Sentinel lymph node biopsy 於乳管原位癌(ductal carcinoma in situ; DCIS)和侵襲癌(invasive breast cancer) 所扮演的角色發表專題研究報告。放射治療(radiation therapy)亦為此次大會討論的重點之一。針對歐美和亞洲(韓、日、中國)就乳癌患者以放射治療處置時之乳房劑量，屏蔽效應和目前放射治療發展和對乳癌患者的預後照顧提出各種評估。AstraZeneca PLC 專職提供一特別演講，就荷爾蒙治療個人化差異為專題，其後 Dr. Sasano 以動情素受體蛋白(estrogen receptor)為標的闡述荷爾蒙治療下的替代性標記，來解釋乳癌荷爾蒙治療預後評估的重要性。另外，Dr. Sasano 也以早期乳癌為專題進行文獻回顧，透過 gene microarray 全面性探討基因表現來做為乳癌患者化療或是臨床預後的判別依據。透過基因剖面來注意臨床處置下，對癌症患者所可能造成不良影響或是限制作出完整的報告。Dr. Mikami，現任京都大學醫學院副教授，以早期乳癌組織上的臨床預後標記說明細胞分裂指標、ER/PR status、p53 表現程度、Ki-67；並且以 DNA ploidy，血管新生、EGFR 表現和細胞凋亡來評估必要性的術前全身性治療（Neoadjuvant therapy）的預測和術後評估標記研究，亦針對 Her-2 在乳癌預後的重要性加以強調。Dr. Roukos 加以指出在未來乳癌患者所發生的原因、治療、術後評估、乃至於術後標記等等的基因體學資料庫更應朝向個人化差異。他認為將已知腫瘤基因和腫瘤抑制基因的遺傳多型性外，

輔佐以基因表現性差異和現今發展 miRNAs 的表現，共同建構出乳癌治療抗療性之整合演算研究基準，將更有助於未來在乳癌診斷及治療的發展。建立予以新的癌症組織、早期癌變和預後的臨床研究觀點闡述癌症分期、發展，以致於乳癌不同的臨床治療預後評估等等的專題報告發表，都為我們研究室對於乳癌細胞生成病理機轉和相關蛋白訊息啟動調控提供了非常寶貴的研究資訊。

會議舉行期間，邀請到現今世界各知名實驗室的主持人，就其專業研究領域給予講演、教育訓練課程和會議研討作廣泛的意見交流等。其研究內容涵蓋有細胞生長訊息傳遞、細胞死亡和老化和基因表現調節、基因受損和修復作用；也探討以化學治療，放射治療和術前全身性治療連結於基因體學、血管生成、腫瘤侵襲和轉移之預後評估、生物資訊學、microRNA、和致癌及抑癌基因的表現和分子流行病學的各項研究報告。針對癌症進行深入淺出的演講、能夠讓更多的研究人員體會到癌症預防、臨床標記和臨床治療的體認，這是非常值得讓國內學界來加以重視的發展。

二、與會心得

此次前往日本京都乳癌國際會議之國內學者亦有許多人，其中來自台大、陽明，北醫、國防、長庚、高雄醫大等各大專院校醫學院及醫學研究中心之癌症研究學者。研究領域以臨床轉譯醫學、臨床組織、臨床治療、分子流行病學和臨床藥理等癌症醫學之相關研究課題。藉由會議舉行期間能夠與國內、外研究先進進行學術交流，尤其是針對亞洲地區人種，包括日、韓、新加坡、中國大陸、香港以及本國對於乳癌腫瘤生成暨癌化發生的成因、基因變異、癌症臨床標記之研究、腫瘤之基因治療和各種癌症致癌感受性危險相關因子之統計分析等各項專題，進行學術經驗的交流、分享彼此研究結果和心得。對於本研究室近年來致力於探討臨床組織檢體的基因體剖面研究、預後研究標記、術後存活率有更清楚的意見回饋。最近，我們亦積極從中草藥中找尋治療乳癌腫瘤的協同藥物，透過這項會議對於我們的研究觀念釐清，有莫大的助益；同時，也可以拓展個人的研究視野和深度，實為難能可貴的進修機會。

本屆日本京都乳癌國際會議中，我們以「miR-21 和 miR-221 為標的基因，探討基因調控表現和乳癌進程發展的分期評估」為題，透過雷射捕獲分析技術(laser capture microdissection) 從病人癌組織和癌相鄰之正常組織分別擷取，再以定量 qRT-PCR 的表現來做為乳癌的分期關聯性分析。藉由 miR-21、miR-200c 和 miR-221 等 microRNA 協同表現的重要性，這些分子標記之表現程度在乳癌組織的高低差異，將被視為乳癌基因體學的前置試驗依據。我們發現 miR-21 與乳癌患者發生腋下淋巴轉移亦有關聯，這些攸關癌化過程的訊息結合臨床組織之分期，進行多變相分析評估結果，將對台灣婦女乳癌之年輕化趨勢和特色，完成初步的研究報告並進行發表。另外，從組織中研究 miRNA 的差異性表現，不但可以了解到基因被逆向

調控於腫瘤抑制基因抑或是致癌基因對癌細胞的進程發展，將這些觀念加以應用研發，可以更有效率地判斷出與癌症生成或癌症治療藥物的機制，這也極可能是未來癌症研究的重要議題。摘要發表期間，同各國學者就乳癌做廣泛的討論並且交換研究心得，實獲益匪淺。現今，在我們的實驗室也陸續投諸許多的人力、心力和資源著手進行此方向的研究，冀望能夠透過對 miRNA 的研究，找出對台灣婦女乳癌發生更有意義的研究標記。相信在未來對台灣地區婦女乳癌的相關研究，將會有更重大的發現。

三、會後建議：

在這次學術研討會中，本人也發現國內、外的研究先進在癌症領域鑽研相當深入。他們除了在自己的專業研究室中指導研究生、博士後研究員，兼具該研究單位對外的合作機會，跨院校及國際合作的對象，可以腦力激盪，並加以截長補短，拓展實驗室以外各項專業技術的進步。除此之外，更結合了臨床與基礎的研究交流，加速解決各項問題，這也是為什麼他們能夠走在學術尖端的理由。在參與此次國際性的研討會議中，也深刻感受到亞洲各國在癌症學術領域的研究也發現到人種因為遺傳的差異，較歐美國家在乳癌的發生和治療策略亦有所不同。深深感受到國內許多研究先進和佼佼者展現出強烈的企圖心，為國內的癌症研究向下紮根，為未來國內乳癌學術研究上的發展而努力。藉由此次學術會議，也拓展我們實驗室同其他國內臨床醫師的合作機會，期望能為國內乳癌防治研究盡綿薄之力。