National Research Program for Genomic Medicine

National Science Council, the Executive Yuan, ROC.

Research Project

基因體醫學國家型科技計畫

研究計畫

Application Status: ☑ **Continuation** ☐ **Revised Application**

Program Classification:

☑ **Genomic Medicine** ☐ **Bioinformatics**

☐ **Proteomics & Structural Genomics** ☐ **ELSI**

Serial Number: 91GMP012-3 (原計畫申請編號)

Title of Component Prject 子計畫名稱	(in Chinese) 中文 人類移形上皮癌之基因表現檔案---尋找與砷有關之致癌機轉及調整化學治療處方
	(in English) 英文 Gene Expression Profiling of Human Transitional Cell Carcinoma---Identifying Arsenic-Related Carcinogenic Mechanism and Tailoring Chemotherapy Regimens
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TABLE OF CONTENTS

Part B.

Progress Report
of Component Project

PROGRAM PROJECT: Component Project __3___(☜請填入子計畫編號)

B2. Progress Report

B2a. Specific Aims

Please state the overall goals of the project, and specific aims, as reviewed and approved by the Study Section and actually awarded. If these specific aims as actually funded did not differ in scope from those actually pursued during the grant period, and if the aims have not been modified, state this. If they have been modified, give the revised aims and the reasons for the modifications.

Specific aims (The aims have not been modified.)

1. Establishing arsenic-related carcinogenic mechanism of human urothelium
   A. Compare gene expression profiles of human urothelial carcinoma (UC) specimens from Blackfoot Disease (BFD) and non-BFD endemic areas in Taiwan by using cDNA microarray.
   B. Formulate possible toxicogenic and carcinogenic pathways of arseniasis by the expressed gene profiles of UCs.
2. Establishing a drug-selecting algorithm for UC
   A. Identify differentially expressed genes in cell lines of varied chemosensitivity and tumor specimens from chemotherapy responders and non-responders.
   B. To build up a drug-selection algorithm for UC chemotherapy and validate the algorithm in a xenograft nude mouse model.

Hypothesis

1. Arsenic-mediated carcinogenesis of human urothelium involves multiple genetic factors which can be delineated by the expressed gene profiles of transitional cell tumors.
2. Human TCC can be classified molecularly by expressed gene profiles which may confer varied sensitivity to chemotherapy.

PROGRAM PROJECT: Component Project __3___(☜請填入子計畫編號)

B2b. Studies and Results

Describe the studies directed toward the specific aims during the current grant period and the results obtained. Indicate the extent to which the work accomplished has successfully met the specific aims. Include negative results. If technical problems were encountered in carrying out this project, describe how your approach was modified.

Establishing arsenic-related carcinogenic mechanism of human urothelium

We have finished the following works:

1. Gene expressions profiles of NTUB1 and NTUB1/As using cDNA microarray have been determined. Gene of 3-fold or higher difference in expression between NTUB1 and NTUB1/As (arsenic trioxide-resistant clone) have been picked up and are thought to play roles in arsenic-related carcinogenesis. (Table 1)

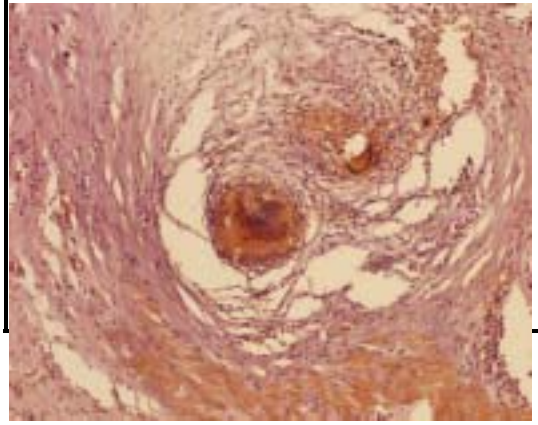
Table 1. Differentially expressed genes: NTUB1/As vs. NTUB1

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)
Nil			
Down-regulated			
1614	0.12	Transcriptional factor	general transcription factor IIB
1750	0.14		stathmin 1/oncoprotein 18
572	0.16	Growth factor or cytokine	pleiotrophin (heparin binding growth factor 8, neurite growth-pron
1204	0.19		ESTs, Moderately similar to 810024E cytochrome oxidase III [H.s
1555	0.2		RAB5 interacting protein 2
1582	0.2		Homo sapiens, clone IMAGE:3677155, mRNA
1286	0.21		ribosomal protein L35
1273	0.24	Phosphatase	protein phosphatase 1, regulatory (inhibitor) subunit 1A

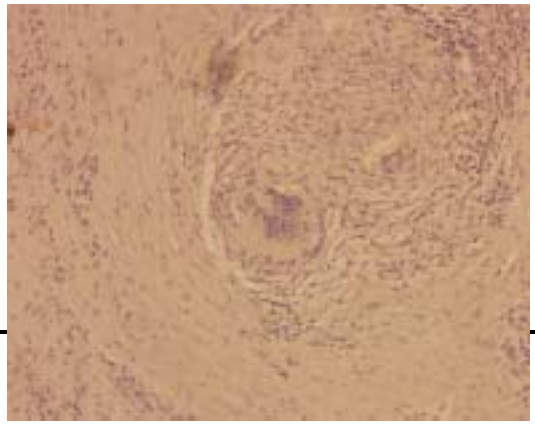
2. The above data will be linked to those from Component Project 2, which used immortalized human urothelial cells (SV-HUC-1 or CRL9520) that were also made resistant to arsenic trioxide by long-term incubation. The common genes responsible for resistance in NTUB1/As and SV-HUC-1/As should be the priority for validation and further in-depth study.

3. One of the up-regulated genes in resistant cell lines, heme oxygenase-1 (HO-1), had been done by real time PCR, but the data are not definitely compatible with those in cDNA microarray. We also used PA3-019 (polyclonal rabbit anti-HO1 antibody) (Affinity BioReagent™) to detect HO-1 protein, which is expressed in all UC tissue samples, regardless of arsenic resisitance; but not in adjacent normal or normal urothelia. We observed that tumors with higher grade and stage or related to arsenic tended to express lower level of HO-1 protein. Whether there is real difference between high-level expression tumors and low-level expression tumors is currently under study.

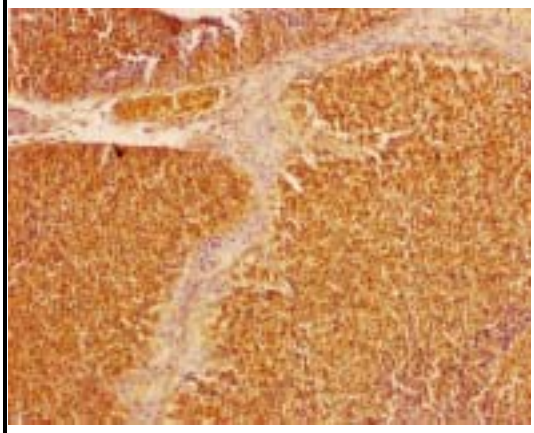
Positive control (Activated macrophages with epithelioid giant cells in TB granuloma)



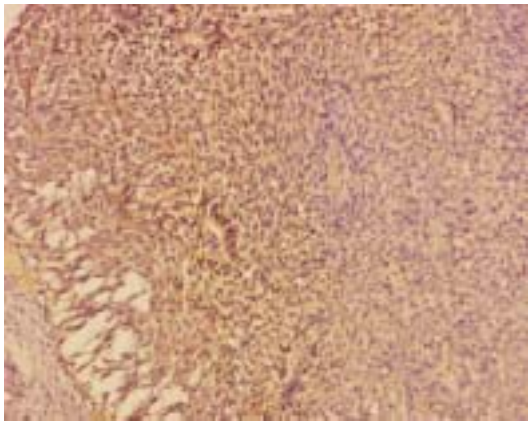
Negative control (Omission of primary antibody)



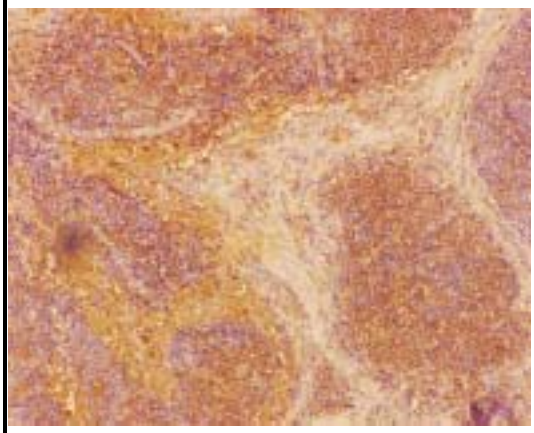
Non-aresenic-related
superficial bladder cancer (Gr I)



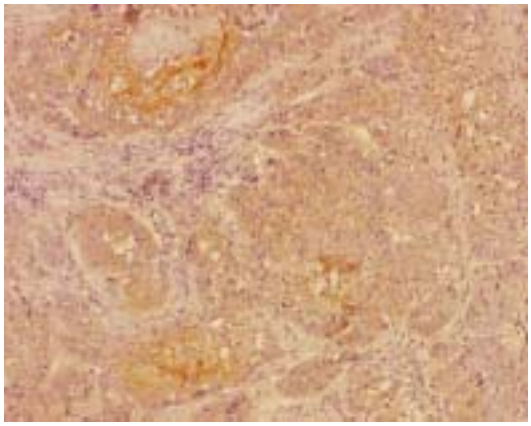
Non-arsenic-related
superficial bladder cancer (Gr II)



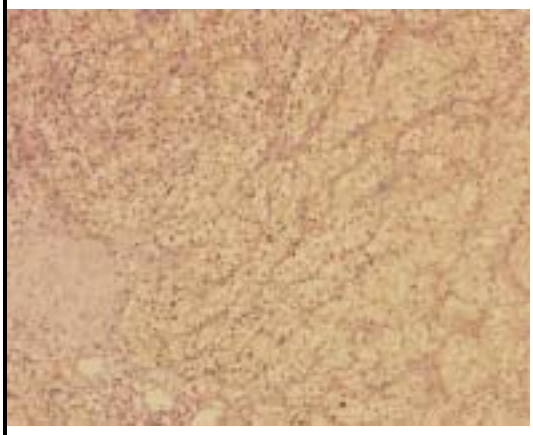
Non-aresenic-related
invasive bladder cancer (Gr III)



Arsenic-related
invasive bladder cancer (Gr III)



Control (renal pelvis urothelium)



It appears that normal urothelium is negative for HO-1 staining. Almost all tumors are positive for HO-1 staining. We are still working on the staining intensity to see if there is differential staining between As-related or unrelated UCs.

4. Tumors and their respective adjacent normal specimens from patients with arsenic-related (n = 15) and unrelated (n = 30) UCs have been collected. Control urothelia (n = 10) from benign urological diseases were also obtained. RNA and cDNA samples from all these tissues have been obtained.

5. Gene expression profiles of some arsenic-related and arsenic-unrelated UCs using cDNA microarray have been done. However, the RNAs from arsenic-related UCs were

of low amount and suboptimal quality, which made microarray results questionable. Linear amplification will be used to amplify the amount of RNA. We will adopt laser capture microdissection and linear amplification to get enough cDNA representing benign urothelia.

6. In the coming grant period, we will continue the cDNA microarray experiment and subsequent data analysis to isolate genes specific for carcinogenesis of arsenic–related UC.

Establishing a drug-selecting algorithm by correlating drug sensitivity with expressed gene profiles in UC

We have finished the following works:

1. In vitro chemosensitivity testing: Six parental sensitive UC cell lines (NTUB1, T24, HTB5, TSGH8301, BFTCC905, and BFTCC909) and 5 daughter resistant UC cell lines (NTUB1/As resistant to arsenic trioxide, NTUB1/G resistant to gemcitabine, NTUB1/P resistant to cisplatin, NTUB1/T resistant to paclitaxel, and T24/A resistant to doxorubicin) have been tested against 8 commonly used chemotherapeutic drugs (cisplatin, doxorubicin, 5-FU, gemcitabine, methotrexate, paclitaxel, vinblastine, and arsenic trioxide). A total of 88 IC_{50} values (11 X 8 = 88) had been obtained. (Table2)

Table 2. IC_{50} of UC cell lines by using the MTT assay

IC_{50}	**Doxorubicin (μM)**	**Cisplatin (μM)**	**Paxlitaxel (μM)**
NTUB1	0.0870±0.0145	1.9365±0.2770	0.0126±0.0007
NTUB1/P (14)	0.4745±0.0384	48.2044±2.1603	0.0736±0.0164
NTUB1/As (0.4)	0.1980±0.0746	1.5965±0.3487	0.0207±0.0044
NTUB1/T (0.005)	0.1087±0.0200	5.6090±0.3118	0.0398±0.0139
T24	0.1292±0.0648	2.3179±0.5110	0.0285±0.0115
T24/A (0.4)	1.8573±0.5125	3.3184±0.7353	0.4254±0.0892
BFTCC905	0.1341±0.0710	0.9093±0.1364	0.0303±0.0119
BFTCC909	0.4662±0.0898	3.4287±0.3935	0.0378±0.0073
IC_{50}	**Gemcitabine (μM)**	**5-FU (μM)**	**As_2O_3 (μM)**
NTUB1	0.1391±0.0169	50.4234±5.7678	0.8191±0.1010
NTUB1/P (14)	2.6541±0.4390	57.8671±1.6749	5.0538±0.4145

NTUB1/As (0.4)	0.2256±0.0725	35.8309±6.8506	0.8514±0.1828
NTUB1/T (0.005)	0.1177±0.0139	42.1262±5.8884	0.7948±0.1216
T24	0.1554±0.0933	72.8369±1.6904	0.6885±0.1263
T24/A (0.4)	1.2149±0.1939	89.9002±5.5595	1.4375±0.0489
BFTCC905	0.5463±0.2633	19.6165±2.3546	0.6817±0.0869
BFTCC909	2.9876±0.2482	40.8998±1.6550	2.0117±0.2492

NTUB1/P, NTUB1/cisplatin-resistant
NTUB1/As, NTUB1/arsenic trioxide-resistant
NTUB1/T, NTUB1/paclitaxel-resistant
T24/A, T24/doxorubicin-resisitant

2. Gene expression profiles of 11 UC cell lines determined by the cDNA microarray technology have been obtained. Genes of 3-fold or higher difference in expression between the 3 most sensitive (mean expression signals) and 3 most resistant UC cell lines have been picked up to be responsible for the chemoresistance. These genes are referred to as "**resistance-related genes**". A total of 79 genes have been identified. (See B2b. Studies and Results (Cont.))

3. Tumor specimens from patients treated with chemotherapy were collected. Pre-chemotherapy tumor RNAs were extracted and cDNAs were made ready for real-time PCR to examine the expression status of the above "resistance-related genes". These tumors had variable responses (complete response [n = 2], partial response [n = 8], stable disease [n = 8], and progressive disease [n = 8]) to chemotherapy containing paclitaxel, 5-fluorouracil, cisplatin, methotrexate, vinblastine, gemcitabine, etc. So theoretically tumor responses can be correlated with the expression status of the "resistance-related genes".

4. A model of predicting the chemosensitivity of a given tumor for each chemotherapeutic agent will be formulated by inputting above data (gene expressions as the independent variables and clinical response as the dependent variable) of about 20 clinical tumor samples. Another set of 20 to 30 clinical tumors will be used to validate the model. However, the difficulty of the following analysis lies in that we use combination chemotherapy in a real clinical situation rather than single agent to treat bladder cancer. We need in-depth bioinformatics support to solve this statistical problem.

Answers to Comment III:

Comment III-1. It is noted that the concerns raised by the previous summary statement have not been addressed in this continuing application. First, part one of this project appears to overlap, aim- and design-wise with component project 2.

Answer to Comment III-1. The component project 3 is mainly dealing with arsenic-related tumor tissues, as compared with the component project 2 that deals with immortalized

human urothelial cells (SV-HUC-1 or CRL9520) that were made resistant to arsenic trioxide by long-term incubation. These 2 component projects pursue the topic from different aspects and are complementary to each other.

Comment III-2. Second, this component project intends to establish a drug-selecting algorithm by correlating drug sensitivity with expressed gene profiles of TCC. Can one equate, at least approximately, the notion of drug sensitivity with the desire to reduce toxicity and improve efficacy? How will the drug sensitivity be measured and have such measures been validated? It is felt that a strong rationale should be provided to assure that the approach for *in vitro* chemosensitivity testing, which is rather complicated with 23 cells subjected to test against 10 agents, will tailor future chemotherapy regimens.

Answer to Comment III-2. This study approach is a novel one which needs verification and validation. If a given tumor is resistant to certain chemotherapeutic agents and sensitive to others, a reasonable strategy to improve treatment efficacy and reduce toxicity is to utilize sensitive drugs but avoid resistant ones. Our strategy is to correlate resistance-related gene expressions of a given tumor and clinical responses to a certain agent. Hopefully, a model of predicting chemosensitivity can be formulated and will be validated by another set of tumors of whom the clinical treatment response and gene expressions by real-time PCR can be obtained.

Comment III-3. The detailed budget request is not provided. Every page after 16 is blank.

Answer to Comment III-3. As requested, the budget request of the third year is attached in the report.

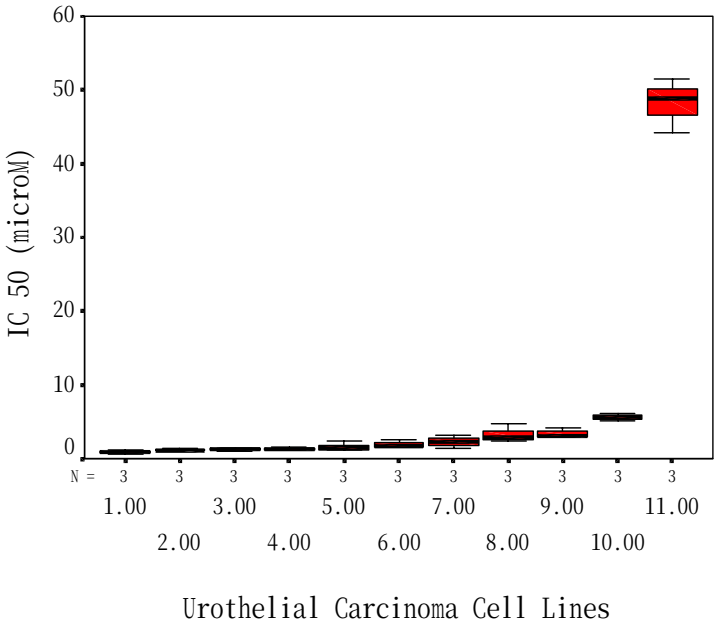
Comment III-4. A major concern raised in previous review was that the proposed approaches for part II are purely exploratory and with the number of units considered (small indeed), it is unclear if meaningful results would be obtained. This concern was not addressed in the progress report.

Answer to Comment III-4. The proposed approaches for part II are not entirely exploratory. For examples, Zajchoski DA, et al. (Cancer Res 2001;61:5168-78) identified gene expression profiles that predict the aggressive behavior of breast cancer cells using 9 weakly invasive and 4 highly invasive breast cancer cell lines (13 cell lines in total). As compared with the 11 parental (presumably chemo-sensitive) cell lines and 12 daughter (presumably chemo-resistant) cell lines used in our study, the power of the study could be as strong as Zajchoski's study. However, we admit that using gene expression profiles of certain cell lines to predict clinical chemosensitivity (or chemoresistance) is exploratory.

B2b. Studies and Results (Cont.)

Cisplatin IC50 ranking list

1 = BFTCC905
2 = TSGH8301
3 = HTB5
4 = NTUB1/G
5 = NTUB1/As
6 = NTUB1
7 = T24
8 = T24/A
9 = BFTCC909
10 = NTUB1/T
11 = NTUB1/P

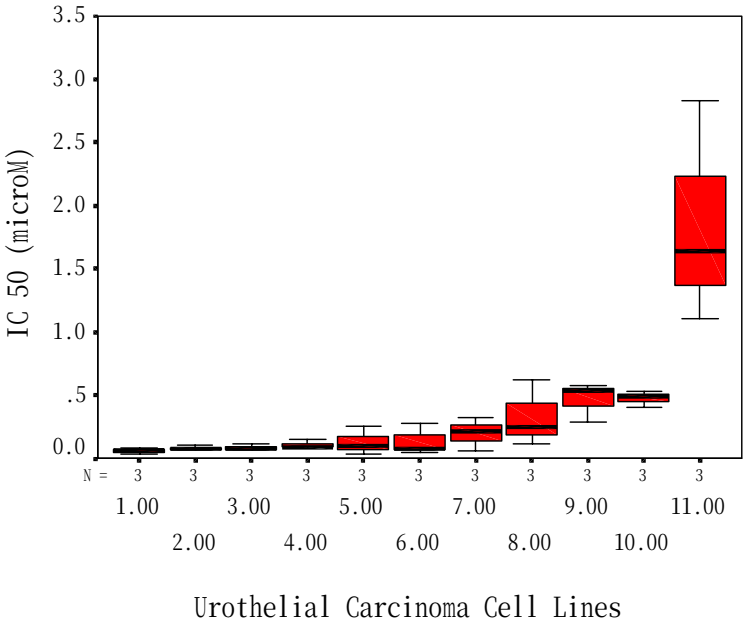


Cisplatin resistance-related genes (cells of the 3 highest IC50s vs cells of the 3 lowest IC50s)

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)	Acc. NO
1987	9.3	Translation	putative translation initiation factor	N91944
878	4.3	Adhesion & ECM	annexin A8	H58091
1299	4.1	Kinase & signaling	histidine triad nucleotide-binding protein	T57556
Down-regulated				
731	0.036	Proteolytic activity	ubiquitin carrier protein E2-C	T86744
1775	0.08	Growth factor or cytokine	antigen identified by monoclonal antibody Ki-67	N52414
2011	0.14		ESTs	
403	0.16	Receptor	activin A receptor, type I	R45384
101	0.22	Miscellaneous	ESTs	N93946
1297	0.22		ribosomal protein L34	
1376	0.24	Kinase & signaling	RAB2, member RAS oncogene family	N20071
194	0.25		similar to RIKEN cDNA 2310040G17 gene	
1827	0.25		small inducible cytokine A5 (RANTES)	

Doxorubicin IC50 ranking list

1 = NTUB1/G
2 = TSGH8301
3 = NTUB1
4 = NTUB1/T
5 = T24
6 = BFTCC905
7 = NTUB1/As
8 = HTB5
9 = BFTCC909
10 = NTUB1/P
11 = T24/A

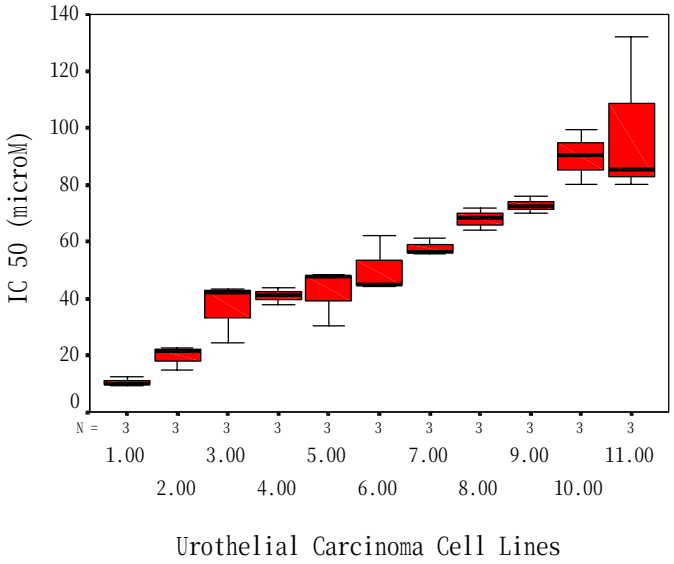


Doxorubicin resistance-related genes (cells of the 3 highest IC50s vs cells of the 3 lowest IC50s)

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 20
885	3.89	Receptor	retinoic acid receptor, beta
1009	3.85	Oncogene & suppressor gene	GRO2 oncogene
813	2.50	Oncogene & suppressor gene	exotosis (multiple) 1
1233	2.39	Kinase & signaling	mitogen-acitvated protein kinase 6
1102	2.06		Homo sapiens mRNA; cDNA DKFZp434F0723 (from clone D
1307	2.05		Human platelet-derived growth factor A chain (PDGFA) gene
Down-regulated			
1809	0.107		chloride channel, calcium activated, family member 4
28	0.195		calnexin
1643	0.197	Kinase & signaling	protein kinase, cAMP-dependent, regulatory, type I, alpha (ti
1982	0.215		tousled-like kinase 1
1542	0.244	Stress protein	secreted protein, acidic, cysteine-rich (osteonectin)

5-Fluorouracil IC50 ranking list

1 = TSGH8301
2 = BFTCC905
3 = NTUB1/As
4 = BFTCC909
5 = NTUB1/T
6 = NTUB1
7 = NTUB1/P
8 = NTUB1/G
9 = T24
10 = T24/A
11 = HTB5

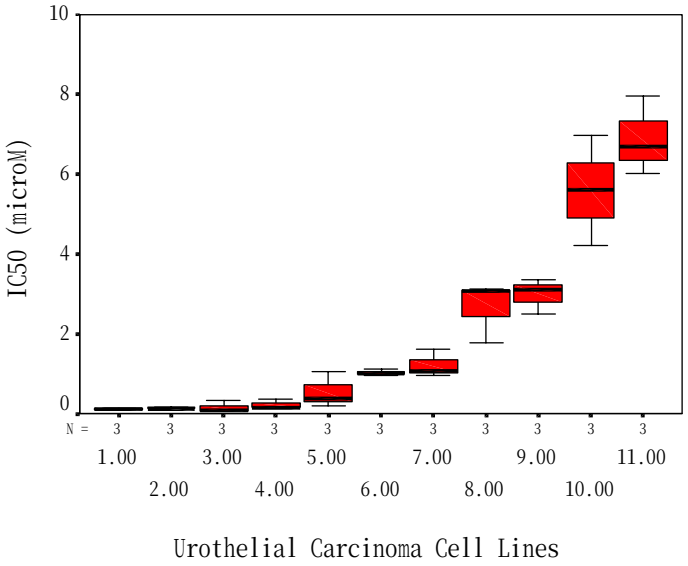


5-FU resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)
13	5.23		indolethylamine N-methyltransferase
134	3.43	Proteolytic acitivity	ubiquitin protein ligase E3A (human papilloma virus E6-associate
1069	3.24		ESTs, Highly similar to unnamed protein product
494	3.12	Growth factor or cytokine	parathyroid hormone-like hormone
480	3.11	Proteolytic acitivity	matrix metalloproteinase 7 (matrilysin, uterine)
Down-regulated			
30	0.14	Adhesion & ECM	integrin, beta 4
1911	0.25		AD023 protein

Gemcitabine IC50 ranking list

1 = NTUB1
2 = T24
3 = NTUB1/T
4 = NTUB1/As
5 = BFTCC905
6 = TSGH8301
7 = T24/A
8 = NTUB1/P
9 = BFTCC909
10 = HTB5
11 = NTUB1/G

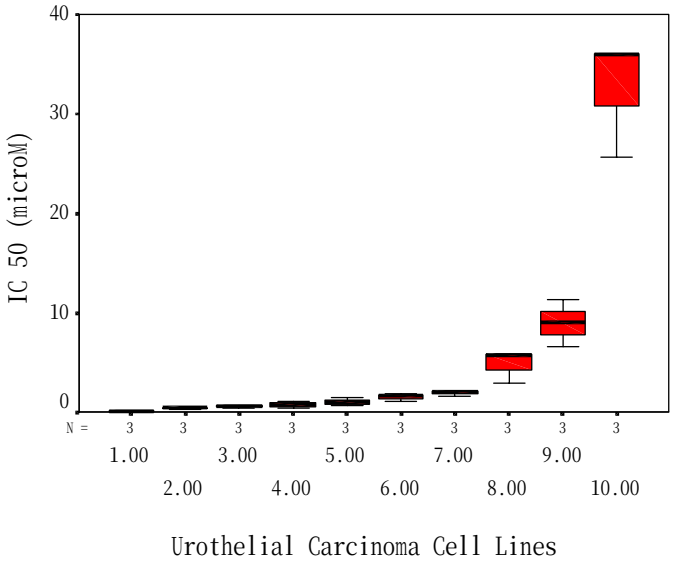


Gemcitabine resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)
1256	18.03	Differentiation	TGF beta receptor associated protein -1
2011	11.16		ESTs
1282	7.13	Kinase & signaling	protein kinase C, zeta
1206	6.94	DNA replication and repair	growth arrest and DNA-damage-inducible, alpha
1263	6.15	Cell-cycle control	CDC-like kinase 2
1693	5.68	unknown	ESTs
1473	5.3		prefoldin 5
1908	5.24		H3 histone, family 3B (H3.3B)
344	4.77		TAF9-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31 kD
1388	4.17		regulated in glioma
786	4.08	Transcriptional factor	adaptor-related protein complex 3, beta 1 subunit
Down-regulated			
111	0.21		phosphatidylinositol glycan, class F
170	0.23	Transcriptional factor	human immunodeficiency virus type I enhancer-binding protein
1013	0.16	Proteolytic activity	proteasome (prosome, macropain) activator subunit 3 (PA28 ga
1190	0.22		eukaryotic translation elongation factor 1 gamma

Methotrexate IC50 ranking list

1 = BFTCC905
3 = NTUB1/As
4 = T24/A
5 = NTUB1/G
6 = NTUB1/T
7 = T24
8 = NTUB1
8 = TSGH8301
9 = NTUB1/P
10 = BFTCC909

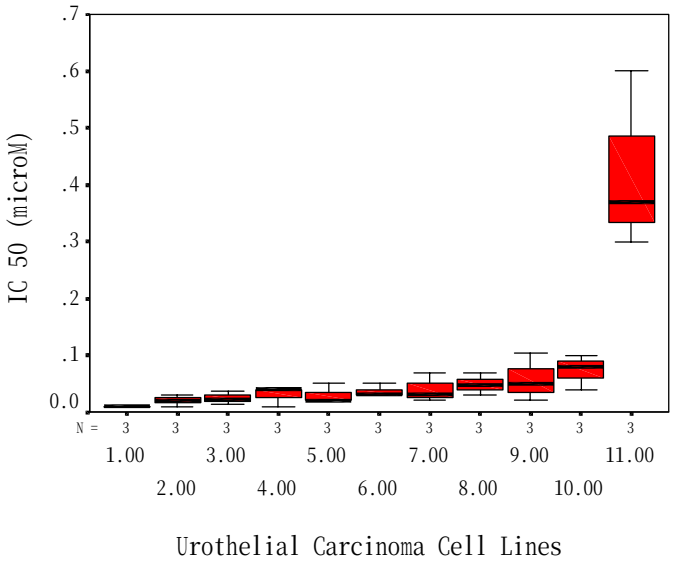


Methotrexate resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31,
154	7.55		CDK2-associated protein 1
464	5.07		ubiquitin B
860	4.48	Oncogene & suppressor gene	oxidase (cytochrome c) assembly 1-like
1117	5.31	Vascular disorder	serine (or cysteine) proteinase inhibitor, clade E (nexin, plasi
1153	4.4	Oncogene & suppressor gene	DEK oncogene (DNA binding)
1301	5.77	Miscellaneous	small nuclear ribonucleoprotein polypeptides B and B1
1526	4.26		hypothetical protein MGC5363
1562	5.07		ESTs
1587	4.05	Oncogene & suppressor gene	sarcoma amplified sequence
1704	4.76		arachidonate 5-lipoxygenase-activating protein
1789	4.09		House keeping GAPDH(200X)
1897	4.51	Translation	putative translation initiation factor
Down-regulated			

Paclitaxel IC50 ranking list

1 = NTUB1
2 = NTUB1/As
3 = NTUB1/G
4 = T24
5 = BFTCC905
6 = BFTCC909
7 = NTUB1/T
8 = TSGH8301
9 = HTB5
10 = NTUB1/P
11 = T24/A

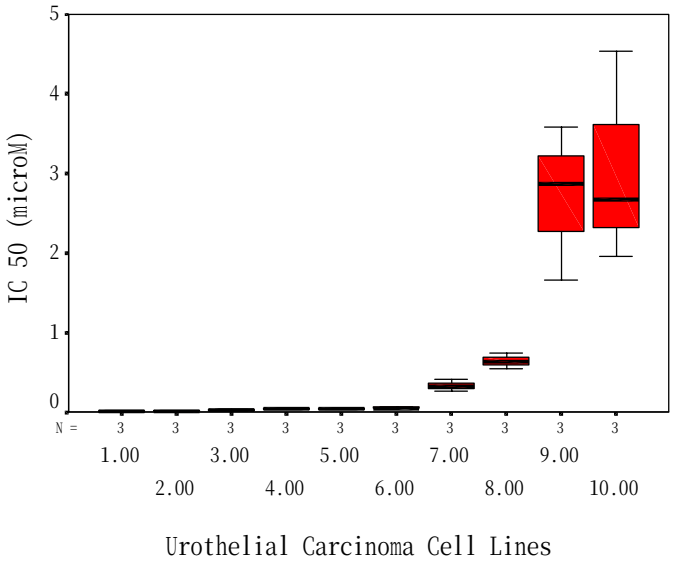


Paclitaxel resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)
885	4.27		Homo sapiens, clone MGC:10965 IMAGE:3633884, mRNA, cor
461	4.25	Kinase & signaling	huntingtin (Huntington disease)
Down-regulated			
1604	0.12		bromodomain adjacent to zinc finger domain, 1A
1720	0.15	DNA replication and repair	eukaryotic translation initiation factor 4A, isoform 2
1524	0.17	Transcriptional factor	HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 1
692	0.21	Kinase & signaling	serine/threonine-protein kinase PRP4 homolog
1531	0.21	House keeping	ribosomal protein S20

Vinblastine IC50 ranking list

1 = NTUB1/As
2 = NTUB1
3 = BFTCC905
4 = NTUB1/T
5 = NTUB1/G
6 = TSGH8301
8 = T24
8 = NTUB1/P
9 = BFTCC909
10 = T24/A

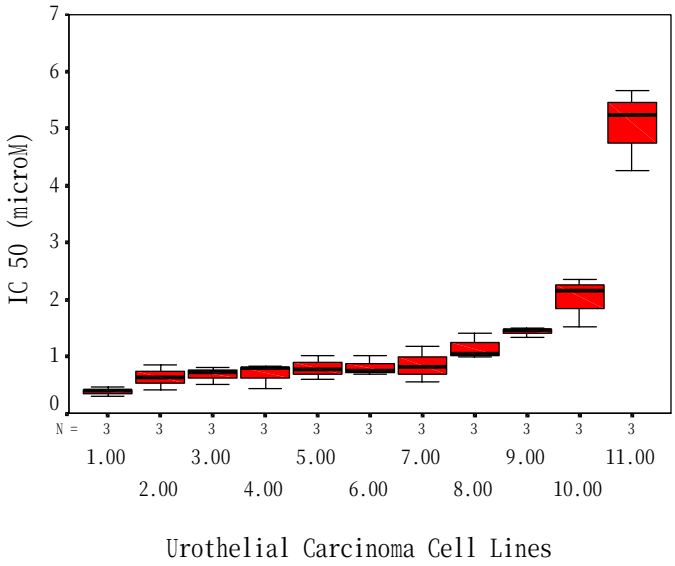


Vinblastine resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2001)
885	10.1		Homo sapiens, clone MGC:10965 IMAGE:3633884, mRNA, complete c
Down-regulated			
1474	0.145	Kinase & signaling	mitogen-activated protein kinase 13
1672	0.186	Adhesion & ECM	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated an
1524	0.187		Egr1

Arsenic trioxide IC50 ranking list

1 = HTB5
2 = NTUB1/G
3 = BFTCC905
4 = T24
5 = NTUB1/T
6 = NTUB1
7 = NTUB1/As
8 = TSGH8301
9 = T24/A
10 = BFTCC909
11 = NTUB1/P



Arsenic trioxide resistance-related genes

Up-regulated	Fold	Gene category	Gene name (revised on Jan.31, 2
Down-regulated			
1775	0.082	Growth factor or cytokine	antigen identified by monoclonal antibody Ki-67
444	0.099		hypothetical protein MGC8721
315	0.111		synaptosomal-associated protein, 23kD
1491	0.119		CDC28 protein kinase 2
2011	0.141		ESTs
1672	0.166	Adhesion & ECM	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associa
302	0.172	Growth factor or cytokine	interleukin 1, beta
1493	0.173		translocase of inner mitochondrial membrane 17 homolog A (yeas
1297	0.183		ribosomal protein L34
131	0.186		ribosomal protein L23
251	0.244		signal transducer and activator of transcription 1, 91kD

PROGRAM PROJECT: Component Project ___3__(☜請填入子計畫編號)

B2c. Personnel

Summarize the personnel involved in the project during the grant period. List the personnel in accordance to the following categories: (1) senior investigators, including visitors; (2) postdoctoral fellows; (3) graduate students; (4) technicians; and (5) other research assistants. Specify for each individual the period of involvement and the percentage commitment of effort.

Position Title	Name Chinese	Name English	% Effort	Job Description or Responsibilities
PI	蒲永孝	Yeong-Shiau Pu	35	Organize and supervise research team, report and manuscript preparation, tissue collection and handling
Co-PI	李德章	Te-Chang Lee	15	Provide gene chips
Co-PI	侯自銓	Tzyh-Chuyan Hour	15	Animal experiments, molecular biology experiments
PhD student	林家齊	Chia-Chi Lin	15	Tissue collection and handling, data analysis
Research associate	官靜儀	Jing-Yi Guan	10	Cell culture, performing microarray assay
Research associate	王榮蓮	Jung-Lien Wang	10	Cell culture, molecular biology experiments

B2d. Projected Timeline

Provide a reasonable timetable for the execution of the work outlined in the project. Highlight appropriate milestones that you might use to target the studies. Indicate technical hurdles that might slow down the execution of the work and discuss any contingencies that you have or might have built in the research plan in anticipation of these difficulties. Do not exceed one page.

A. Arsenic-mediated carcinogenic mechanisms

First Year (2002/05 to 2003/04)

1. Collection and extraction of tumor RNA of human UC tissues

Second Year (2003/05 to 2004/04)

2. cDNA microarray study (primary gene chip)
3. Analyze microarray results and select differentially expressed genes
4. Q-PCR to confirm the expression status.
5. Construct secondary gene chips that contain the above differentially expressed genes.
6. LCM and Linear amplification of tumor RNA of human UC tissues (30 cases for each of the two groups of UC specimens).

Third Year (2004/05 to 2005/04)

7. cDNA microarray (secondary gene chip) study.
8. Q-PCR to confirm the expression status.
9. Functional study of significant unknown genes found in the study.
10. Link data with those from Component Project 2, which uses human urothelium cells (CRL9520) with long-term treatment of MMA(III), DMA(III), and arsenic trioxide.
11. Preparation of project reports and manuscripts to be published.

B. To establish a drug-selecting algorithm by correlating drug sensitivity with expressed gene profiles in TCC

First Year (2002/05 to 2003/04)

1. Establish chemosensitivity profiles of 23 UC cell lines (sensitive and resistant). Organize the cell line list by the order of chemosensitivity.
2. Extract RNA for cDNA microarray study.

Second Year (2003/05 to 2004/04)

3. Q-PCR to confirm the expression status of these genes in cells.
4. Construct the drug-selecting algorithm

Third Year (2004/05 to 2005/04)

5. Validate and modify the drug-selecting algorithm
6. Clinical tumor study: LCM and linear RNA amplification of clinical tumor samples for cDNA microarray study.
7. Correlating chemotherapy response with data of microarray study.
8. Validate and modify the drug-selecting algorithm
9. Preparation of project reports and manuscripts to be published.

PROGRAM PROJECT: Component Project ___3__(☜請填入子計畫編號)

B2e. Publications (Optional)

List the title and complete references (author(s), journal or book, year, page number) of all publications resulting from studies supported by the project. List the publications for the project in accordance to the following categories: (1) manuscripts published and accepted for publications; (2) manuscripts submitted and under review; (3) manuscripts under preparation; and (4) conference proceedings. Provide one copy of each publication not previously reported to the National Science Council in the Appendix.

Conference Proceedings

1. Gene expression profiling of human urothelial carcinoma – identifying arsenic-related carcinogenic mechanism and tailoring chemotherapy regimens. Chia-Chi Lin, et al. Proc Annual Meeting of Taiwan Urology Association 2003 B05. (Aug 30, 2003)

B2f. Patents (Optional)

List all inventions disclosed, patents filed, and patents granted.

PROGRAM PROJECT: Component Project __3___(☜請填入子計畫編號)

B3. Summary of the Modified Budgets (Optional)

B3a. Background and Statement (including literature cited)

Please describe the background leading to the present **revised** project and discuss the potential difficulties and limitations of the previously proposed application. List all major changes in the budget and the personnel, and provide a justification for the change. State concisely the importance of the requested revision or supplement by relating the specific aims to the broad, long-term objectives, as well as the overall goals of the project.

We had changed the master student to Jen-Mei Lee. Because the prior master student had graduated and went to military service.

PROGRAM PROJECT: Component Project _____ (☜請填入子計畫編號)

B3b. Summary Budget Requested in NT dollar (in NT dollars: 1USD = 34 NTD)

Budget Categories	1st Year (granted) (__y__m __y__m)	2nd Year (granted) (__y__m __y _m)	3rd Year (requested) (__y__m __y__m)	Remarks for Changes
1. Personnel (Form B3d)				
2. Equipment (Form B3e)				
3. Travel to Overseas /Mainland (Form B3f)				
4. Attending International Conferences (Form B3g)				
5. Others* (Form B3h)				
6. Overhead**				
7 .Use of Core Facilities (Form B3i)				
8. Bonus for the PI***				
9. Total****				
10 Postdoctoral Fellow (The Number of Person)				
Total for entire project period:	NT$			
Other Personnel and Supplemental Request				
National High Computing Center (Quota)				
Precision Instrument Center (Quota)				

Other Research support

Funding Agency	Items	Budget	Facial Year

Official Signing for Applicant Organization：_____ Date：_____

* Including Consumables, Miscellaneous, Animal Study, Travel Expense, Publication Costs, and Maintenance

** Overhead = (Sum of Item 1 to 5) x 8%

*** The "Bonus for the PI" is NT$120,000 per year. However, if the PI has more than one NSC grant application this year, he or she can only request the bonus in one application.

**** Total = sum of item 1 to 8 (use of core facilities should be included)

PROGRAM PROJECT: Component Project _____ (☜請填入子計畫編號)

B3c. Postdoctoral Fellows Requested

Please justify the need of requested postdoctoral fellows in terms of numbers and disciplines (or areas of specialty).

PROGRAM PROJECT: Component Project _____(☜請填入子計畫編號)

B3d. Detailed Budget for Personnel (3rd Year, requested)

Class/Grade	Name	Salary (NT$)			Role in Project
	Chinese English	Monthly	Annual	Insurances (Annual)	
PhD Student	林家齊 Chia-Chi Lin	20,000	240,000		Tissue collection and handling, data analysis
Master Student	李貞妹 Jen-Mei Lee	8,000	96,000		Microarray assay, molecular biology experiments
Bachelor/ Ninth year	官靜儀 Jing-Yi Guan	36,300	490,050	40,992	Laser capture microdissection, performing microarray assay (Insurance coverage per year)
Bachelor/ Ninth year	王榮蓮 Jung-Lien Wang	36,300	490,050	40,992	Cell culture, RNA extraction and Amplification, Q-PCR, molecular biology experiments
Subtotal				1,398,084	
Total					

*** Insurance, the year-end bonus, and other fringe benefits should be included in the budget for the personnel.**

PROGRAM PROJECT: Component Project _____(☜請填入子計畫編號)

B3e. Detailed Budget Requested for Equipments in NT dollars

Equipment	Function and Justification	1st year (granted)	2nd year (requested)	3rd year (planned)
Subtotals for each year: NT$				
Total for entire project period: NT$				

PROGRAM PROJECT: Component Project _____(☜請填入子計畫編號)

B3f. Detailed Budget Requested for Travel to Overseas in NT dollars

*** Schedule of the travel**

*** Detailed Budget**

Name	Item/Budget	Description	1st Year (granted)	2nd Year (requested)	3rd year (planned)
	1. Transportation/ 2. Living expense/ 3. Others/ …				
	1. 2. 3. …				
Subtotals for each year: NT$					
Total for entire project period: NT$					

B3g. Detailed Budget Requested for Attending Conferences in NT dollars

*** Description of the period and location of the conference to be attended.**

*** Detailed Budget**

Name	Item/Budget	Description	1st Year (granted)	2nd Year (requested)	3rd year (planned)
	1. Transportation/ 2. Living expense/ 3. Registration fee/ 4. Others/ …				
	1. 2. 3. 4. …				
Subtotals for each year: NT$					
Total for entire project period: NT$					

B3h. Detailed Budget Requested for Other Categories

(Supplies, Consumables, Maintenance, Travel, Experimental Animal, Publication Costs, and Miscellaneous)

Year	Item	Description	Unit	Quantity	Price	Total	Remark
					NT$	NT$	
2nd or 3rd Year							
	Total						

*** Please describe year by year.**

PROGRAM PROJECT: Component Project _____(☜請填入子計畫編號)

B3i. Use of Core Facilities Requested

Check the major core facilities that you intend to use as part of the work proposed in the component project, and include the costs of supplies and other consumables anticipated from these uses. (Please see website http://www.sinica.edu.tw/~asgpp/ for provided items and prices.)

Core Facilities	1st Year (granted)	2nd Year (requested)	3rd Year (planned)	Justification
A1. ENU Mutagenesis and Phenotyping Core Facility A2. Functional and Micro-Magnetic Resonance Imaging Center A3. PET Gene Probe Core B1. Clinical core for Genomic Medicine Research B2. Identifying Hereditary Cancers in Taiwan C1. National High Throughput Facility for Physical Mapping and DNA Sequencing C2. High-Throughput Genotyping Core Facility C3. A Microarray and Gene Expression Analysis Core Facility C4. High Throughput Microarray Anlaysis C5. Microarray Core Facility for Genomic Medicine D1. High Throughput Recombinant Protein Production Core D2. High Throughput and High Capacity Core Facilities for Proteomic Research, Service and Technology Development D3. High-Throughput Protein X-ray Crystallography Core Facility D4. Use of Synchrontron Radition Facilities e.g. SRRC, Spring 8, ALS D5. High-Field Biomacromolecular Solution NMR Core Facility E1. Bioinformatics: Computing Cluster and Application				
Total				

PROGRAM PROJECT: Component Project _____(☜請填入子計畫編號)

B3j. Biographical Sketches of New Personnel

姓　　名	(in Chinese)	ID No. (身份証或護照字號)	
Name (in Print)	(in English)	Date of Birth	
Signature		Sex	□ Male □ Female
Education (Degree, Year, Field of Study)			
Institution and Location			
Research, Professional Experiences and Publication list			

Appendix

Component Project: 3

B6a. Budget Requested for Entire Proposed Project Period (in NT$)

Budget Categories	1st Year From 5 /2002 to 4 /2003 (mm/yy)	2nd Year From 5 /2003 to 4 /2004 (mm/yy)	3rd Year From 5 /2004 to 4 /2005 (mm/yy)
Personnel	1,247,760	1,247,760	1,247,760
Equipment	0	0	0
Travel to Overseas or Mainland China	0	0	0
Attend International Conference	93,944	92,720	90,760
Others*	1,380,000	1,400,000	1,300,000
Overhead (8%)	217,737	219,239	211,082
Total	3,239,441	3,259,719	3,149,602
Usage of Core Facilities	300,000	300,000	300,000
Postdoctoral Fellow (Person)	0	0	0
PhD Graduate Fellowship (Person)	1	1	1

Sources of Other Support

Name of Agency	Item	Amount (NT$)	Year

* Including Consumables, Miscellaneous, Animal Study, Travel Expense, Publication Fee, and Maintenance

Component Project: 3

B6b. Major Personnel

Position Title*	Name Chinese	Name English	% Effort	Role in Project
PI	蒲永孝	Yeong-Shiau Pu	35	Organize and supervise research team, report and manuscript preparation, tissue collection and handling
Co-PI	李德章	Te-Chang Lee	15	Provide gene chips
Co-PI	侯自銓	Tzyh-Chuyan Hour	15	Animal experiments, molecular biology experiments
PhD student	林家齊	Chia-Chi Lin	15	Tissue collection and handling, Data analysis, Sequencing, Q-PCR
Research associate	官靜儀	Jing-Yi Guan	10	Laser capture microdissection, performing microarray assay
Research associate	王榮蓮	Jung-Lien Wang	10	Cell culture, molecular biology experiments

List of Grants for the last three year of Principle Investigator, Co- Principle Investigator, Research Associates and Postdoctoral Fellows

Name of Personnel	Title of Project	Role in Project	Project period (mm/yy)	Funding Agency
Yeong-Shiau Pu	**Expression and prognostic value of a novel tumor suppressor, C-CAM in human prostate cancer**	PI	08/98~ 07/99	National Science Council
Yeong-Shiau Pu	**Cadmium, prostate specific antigen and prostate cancer (I & II)**	PI	08/98~ 07/00	National Science Council
Yeong-Shiau Pu	**Searching Novel Treatment for Hormone-Refractory Prostate Cancer (I, II & III)**	PI	08/99~ 07/02	National Science Council
Yeong-Shiau Pu	**Exposure of Urothelial Cells to Inorganic Arsenic and Drug Resistance Mechanisms in Arsenic-Related Urothelial Cancer (I, II & III)**	PI	08/99~ 07/02	National Science Council
Yeong-Shiau Pu	**A Molecular Epidemiological Study of Urinary Transitional Carcinoma in the Southwestern Area of Taiwan (I, II & III)**	Co-PI	08/99~ 07/02	National Science Council
Yeong-Shiau Pu	**Characterization of Molecular Mechanisms of Androgen-Independent Growth of Prostate Cancer Cells**	PI	09/99~ 12/00	National Taiwan University Hospital
Te-Chang Lee	Mechanisms of Arsenite-Mediated Heme Oxygenase Expression in Arsenic-resistant Cells	PI	8/96 7/99	NSC

Te-Chang Lee	Micronucleus Frequency as a Cytogenetic Marker for Arsenic Exposure in Humans and its Inhibition by Antioxidant	Co-PI	7/98 6/99	Academia Sinica
Te-Chang Lee	Genetic Toxicology: Study of arsenic-induced alterations of gene expression in human cells	PI	8/98 7/01	NSC
Te-Chang Lee	A Study on Genetic Susceptibility to Arsenic-induced Skin Cancer	Co-PI	7/99 12/00	Academia Sinica
Te-Chang Lee	Study of Chromosome segregation disturbance by inorganic arsenic	PI	8/99 7/02	NSC
Te-Chang Lee	Alterations of Gene Expression Profiles in Arsenic-Induced Urinary Transition Cell Carcinoma	PI	8/01 7/02	NSC
Tzyh-Chyuan Hour	Therapeutic Roles and Molecular Mechanisms of Antioxidants in Prostate Cancer	PI	12/01 11/02	NSC

*** Personnel in project can be classified into Principle Investigator (PI), Co-PI, Research Associates and Postdoctoral Fellow**

Component Project: 3

B6c. Postdoctoral Fellow*

Nil

*Please justify the requirement and disciplines of postdoctoral fellow.

Component Project: 3

B6d. Budget for Personnel

Class/Grade	Name	Amount Requested (NT$)		Role in Project
		Monthly	Annual	
First year (05/02-04/03)				
Bachelor/ Third year	Jing-Yi Guan	31,200	421,200 (+34,680)	Laser capture microdissection, performing microarray assay (Insurance coverage per year)
Bachelor/ Third year	Jung-Lien Wang	31,200	421,200 (+34,680)	Cell culture, RNA extraction and Amplification, Q-PCR, molecular biology experiments
Ph.D student	Chia-Chi Lin	20,000	240,000	Conduct experiments
MS student	To be hired	8,000	96,000	Conduct experiments
Subtotal			**1,247,760**	
Second year (05/03-047/04)				
Bachelor/ Third year	Jing-Yi Guan	31,200	421,200 (+34,680)	Laser capture microdissection, performing microarray assay
Bachelor/ Third year	Jung-Lien Wang	31,200	421,200 (+34,680)	Cell culture, RNA extraction and Amplification, Q-PCR, molecular biology experiments
Ph.D student	Chia-Chi Lin	20,000	240,000	Conduct experiments
MS student	To be hired	8,000	96,000	Conduct experiments
Subtotal			**1,247,760**	
Third year (05/04-04/05)				
Bachelor/ Third year	Jing-Yi Guan	31,200	421,200 (+34,680)	Laser capture microdissection, performing microarray assay
Bachelor/ Third year	Jung-Lien Wang	31,200	421,200 (+34,680)	Cell culture, RNA extraction and Amplification, Q-PCR, molecular biology experiments
Ph.D student	Chia-Chi Lin	20,000	240,000	Conduct experiments
MS student	To be hired	8,000	96,000	Conduct experiments
Subtotal			**1,247,760**	
Total			**3,743,280**	

Component Project: 3

B6e. Biographical Sketch of Research Associates

Name		Jing-Yi Guan			Jung-Lien Wang		
Birthday (mm/dd/yy)		09/29/1971 (mm/dd/yy)	Sex	( ) Male (✓) Female	04/05/1960 (mm/dd/yy)	Sex	( ) Male (✓) Female
Full-time Research Assistant		( ) High School ( ) Junior College (✓) Bachelor ( ) Master			( ) High School ( ) Junior College (✓) Bachelor ( ) Master		
Research Compensate		( ) Lecturer ( ) Teaching Assistant			( ) Lecturer ( ) Teaching Assistant		
Period		From 8 / 01 To 7 / 02 (mm/yy)			From 08 / 01 To 07 / 02 (mm/yy)		
Monthly Amount/Award		31,200 NTD			31,200 NTD		
Full-time Research Assistant	Highest Degree	Public Health Department, Taipei Medical University			Graduate From : Department of Horticulture, National Taiwan University		
	Period	From 09 / 90 To 06 / 94 (mm/yy)			From 09 / 79 To 06 / 83 (mm/yy)		
Doctor/Master Student		Date of Entrance: / (mm/yy) Name of School:			Date of Entrance: / (mm/yy) Name of School:		
Lecturer / TA		Date of Employment: / (mm/yy)			Date of Employment: / (mm/yy)		
Research Experience of Full-time Research Assistant	Title	1. Chemopreventive effect of curcumin on bladder cancer---in vitro model			1. Five-Year Project for Establishment of a Cancer in National Taiwan University Medical College (Part I) and Research Projects of the Center for 1994-5 (Part II)		
	Series No.	NSC86-2314-B-002-117			DOH 84-HR-201		
	Period	From 08 / 96 To 07 / 97 (mm/yy)			From 07 / 94 To 06 / 95 (mm/yy)		
	Title	2. Exploring the role of cytokine IL-6 in the prostate carcinoma			2. PCR Quantitation of lung cancer mucin gene expression and correlation with prognosis of patients		
	Series No.	NSC-87-2314-B-002-324			NSC 85-2331-B-002-021		
	Period	From 08 / 97 To 07 / 98 (mm/yy)			From 08 / 95 To 07 / 96 (mm/yy)		
	Title	3. Expression and prognostic value of a novel tumor suppressor, C-CAM in human prostate cancer			3. The formation of myotendinous junction		
	Series No.	NSC88-2314-B-002-188			NSC 89-2314-B-002-465		
	Period	From 08 / 98 To 07 / 99 (mm/yy)			From 08 / 00 To 07 / 01 (mm/yy)		
	Title	4. Searching Novel Treatment for Hormone-Refractory Prostate Cancer (I, II & III)			4.		
	Series No.	NSC89-2314-B-002-149 NSC90-2314-B-002-586 NSC91-2314-B-002-213					
	Period	From 08 / 99 To 07 / 02 (mm/yy)			From / To / (mm/yy)		

Component Project: _3

B6f. Budget for Equipments

Minor and Major Equipment Needed for the Project and Justification. List all equipment (unit cost exceeding NT$ 35,000) required to carry out the project. Identify all items exceeding NT$ 500,000 as major equipment and provide a justification of the need for each of these items, as well as an estimate of the percentage of usage of the equipment item as part of the project. If the latter estimate is significantly less than 25%, consider sharing the use of the equipment item with other component projects.

Year	Description of Equipment	Cost	Check if Major Equipment	Check if Shared Use
01 or **02** or **03**	**Nil**			
	Total			

Form P016 / **(Page No./Total Page)**

*** Please describe year by year.**

Component Project: 3

B6g. Budget for Other Categories

(Miscellaneous, Maintenance, Travel, Animal Study, Publication Fee, and Consumables)

Year	Item	Description	Unit	Quantity	Price	Total	Remark
					NT$	NT$	
01	Chemotherapeutic drugs, chemicals, buffers and reagents	Cellular cytotoxicity assay	set	10	10,000	100,000	
	Cell culture	Media, antibiotics, CO_2 serum, dishes, LN2	set	10	20,000	200,000	
	DNA probes, primers and reagents	for PCR & RT-PCR, Q-PCR, Fluorescence tags, etc	set	8	35,000	280,000	
	cDNA microarray (membranes)		set	10	20,000	200,000	
	LCM analysis	RNA extraction, Gene chips, hybridization kit, etc	set	20	15,000	300,000	
	Plasticwares, glassware	Microdissection, linear RNA amplification	box	20	10,000	200,000	
	Miscellaneous	Pipette, Dropper, Tips, plates, eppendorf centrifuge tubes etc				60,000	
		Stationery, Xerox, blank CD, software, publication fee, computer usage, maintenance of equipment, etc.				20,000	
	Domestic traveling for meetings	Domestic workshop & symposium				20,000	
	Post fee, long-distance calls						
	Total					1,380,000	

Component Project: 3

B6g. Budget for Other Categories

(Miscellaneous, Maintenance, Travel, Animal Study, Publication Fee, and Consumables)

Year	Item	Description	Unit	Quantity	Price	Total	Remark
					NT$	NT$	
02	Cell culture for functional study of unknown genes	Media, antibiotics, CO_2 serum, dishes, LN2	Set	5	20,000	100,000	
	DNA probes, primers and reagents	for for PCR & RT-PCR, Q-PCR, Fluorescence tags, etc	set	10	20,000	200,000	
	cDNA microarray (membranes)	RNA extraction, Gene chips, hybridization kit, etc	set	15	20,000	300,000	
	LCM analysis	Microdissection, linear mRNA amplification	set	20	15,000	300,000	
	Plasticwares, glassware	Pipette, Dropper, Tips, plates, eppendorf centrifuge tubes etc	box	15	10,000	150,000	
	Animals and raise expense	Nude mice xenograft experiment	One	50	5,000	250,000	
	Miscellaneous	Stationery, Xerox, blank CD, software, publication fee, computer usage, maintenance of equipment, etc.				60,000	
	Domestic traveling for meetings	Domestic workshop & symposium				20,000	
	Post fee, long-distance calls					20,000	

	Total					1,400,000	

Component Project: 3

B6g. Budget for Other Categories

(Miscellaneous, Maintenance, Travel, Animal Study, Publication Fee, and Consumables)

Year	Item	Description	Unit	Quantity	Price	Total	Remark
					NT$	NT$	
03	Cell culture	Media, antibiotics, CO_2 serum, dishes, LN2	set	10	20,000	200,000	
	DNA probes, primers and reagents	for PCR & RT-PCR, Q-PCR, Fluorescence tags, etc	set	8	35,000	280,000	
	cDNA microarray (membranes)	RNA extraction, Gene chips, hybridization kit, etc	set	10	20,000	200,000	
	LCM analysis	Microdissection, linear RNA amplification	set	20	15,000	300,000	
	Plasticwares, glassware	Pipette, Dropper, Tips, plates, eppendorf centrifuge tubes etc	box	20	10,000	200,000	
	Miscellaneous	Stationery, Xerox, blank CD, software, publication fee, computer usage, maintenance of equipment, etc.				80,000	
	Domestic traveling for meetings	Domestic workshop & symposium				20,000	
	Post fee, long-distance calls					20,000	

	Total					1,300,000	

Form P017 / (Page No./Total Page)

***Please describe year by year.**

Component Project: 3

B6h. Use of Core Facilities Planned.

Check the major core facilities that you intend to use as part of the work proposed in the component project, and include the costs of supplies and other consumables anticipated from these uses.

Year	Check Core(s) Needed	Core Facilities	Consumables Needed Amounts (in NT$)	Justification
01		**1.** DNA Sequencing Facilities	200,000	
		2. Oligo synthesis	60,000	
		3. Bioinformatics and data mining	40,000	
		Subtotal	300,000	
02				
		1. DNA Sequencing Facilities	120,000	
		2. Microarray construction	80,000	
		3. Oligo synthesis	50,000	
		4. Bioinformatics and data mining	50,000	
03		**Subtotal**	300,000	
		1. DNA Sequencing Facilities	120,000	
		2. Microarray construction	80,000	
		3. Oligo synthesis	50,000	
		4. Bioinformatics and data mining	50,000	
		Subtotal	300,000	
Total			900,000	

*** Please describe year by year.**

Component Project: 3

B6i. Travel to Overseas

Name	Role on the project	Categories	Schedule of the travel
NIL			

Budget		
Items	**Budget**	**Description**
Traffic		
Living		
Others		
Totals		

* **Categories:** including experiments, research, or investigation.

*** Please describe year by year.**

Component Project: 3

B6j. Attending Conferences

Please describe the period and location of the attending conference, and the budget.

I.

1. Conference: 94th annual meeting of American Association for Cancer Research
2. Time: April 5-9, 2003
3. Location: Toronto, Ontario, Canada

Bugget:

1. Traffic: 39,000
2. Living: 37,944 (6,324/day X 6 days)
3. Other: 17,000 (registration rate)
4. **Subtotal: 93,944 (first year)**

II.

1. Conference: 95th annual meeting of American Association for Cancer Research
2. Time: March 27-31, 2004
3. Location: Orlando, FL, USA

Bugget:

1. Traffic: 39,000
2. Living: 36,720 (6,120/day X 6 days)
3. Other: 17,000 (registration rate)
4. **Subtotal: 92,720 (second year)**

III.

1. Conference: 96th annual meeting of American Association for Cancer Research
2. Time: April 16-20, 2005
3. Location: Anaheim, CA, USA

Bugget:

1. Traffic: 35,000
2. Living: 38,760 (6,460/day X 6 days)
3. Other: 17,000 (registration rate)
4. **Subtotal: 90,760 (third year)**

Total: 277,424

The conferences that the applicant attended during the last 3 years. Please describe the name, the time, the location of the conference, and the sources of support.

(I)

1. Conference: 92nd annual meeting of American Association for Cancer Research
2. Time: March 24-28, 2001
3. Location: New Orleans, LA, USA
4. Sources of support: National Science Council

(II)

1. Conference: 91st annual meeting of American Association for Cancer Research
2. Time: April 1-5, 2000
3. Location: San Francisco, CA, USA
4. Sources of support: National Science Council

*** Please describe year by year.**