行政院國家科學委員會專題研究計畫　期中進度報告

粒線體融合素/GTP 胺在非小細胞肺癌病人中過度表現的預後意義(2/3)

計畫類別： 個別型計畫
計畫編號： NSC92-2314-B-039-013-
執行期間： 92 年 08 月 01 日至 93 年 07 月 31 日
執行單位： 中國醫藥大學醫學系

計畫主持人： 許南榮

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 93 年 6 月 1 日

行政院國家科學委員會補助專題研究計畫☑期中進度報告

粒線體融合素/GTP 酶在非小細胞肺癌病人中過度表現的預後意義

Prognostic significance of mitofusin/GTPase overexpression in patients with non-small cell lung cancer

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC91－2314－B－039－018－

執行期間：　91 年 08 月 01 日至　94 年 07 月 31 日

計畫主持人：　許南榮

計畫參與人員：周寬基 (kcchow@dragon.nchu.edu.tw)

姜淑芬

林美瑤

成果報告類型：☑ 精簡報告

執行單位：中國醫藥大學附設醫院 胸腔外科與

國立中興大學 生物醫學研究所

中　華　民　國　93 年　5 月　31 日

研究計畫上所列預期完成之工作項目：

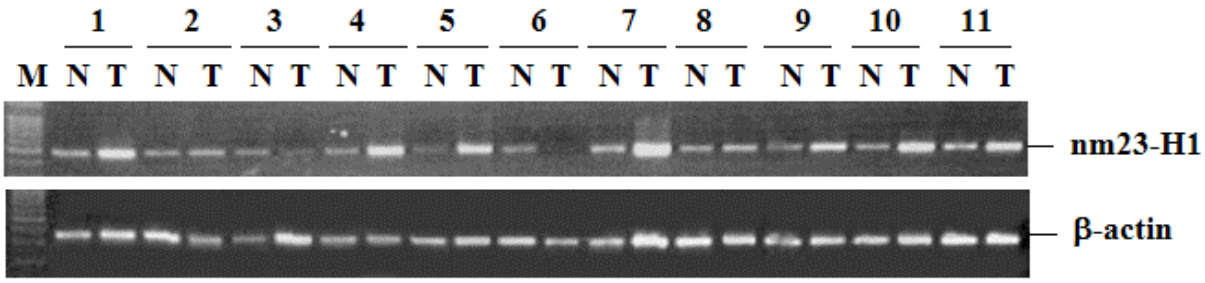
第二年，我們所要完成之工作項目 (92 年 08 月 01 日至　93 年 07 月 31 日)：

(1) To determine gene expressions of mitofusin/GTPases, dihydrodiol dehydrogenases, cyclooxygenases, bcl-2, and nm23 in different lung cancer specimens, by using immunoblotting, immunocytochemistry, immunohistochemistry, and *in situ* hybridization, respectively;

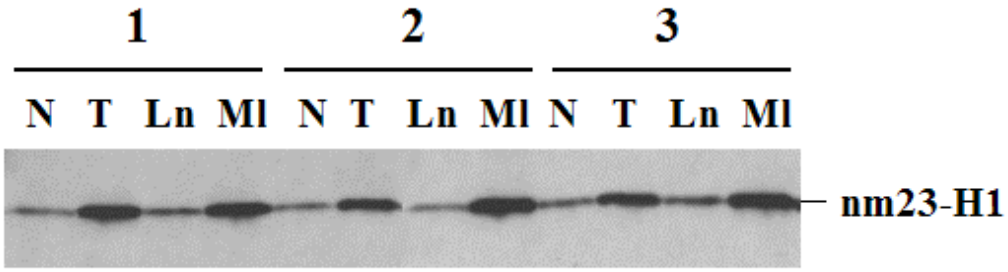
(2) To determine mitofusin/GTPase expression in different lung cancer cell lines, by using immunoblotting, immunocytochemistry, *in situ* hybridization and RT-PCR

進度結果

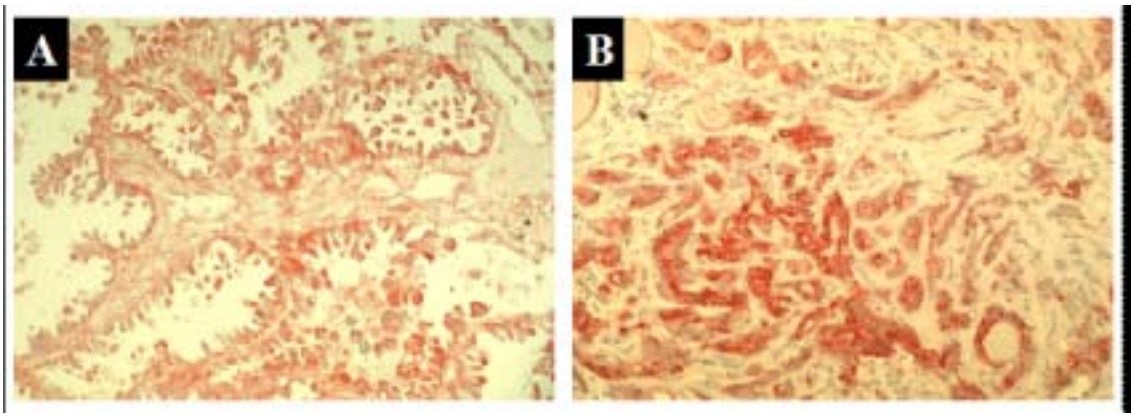
1. Overexpression of nm23-H1 was detected in 54% (6/11) of lung cancer biopsies by RT-PCR



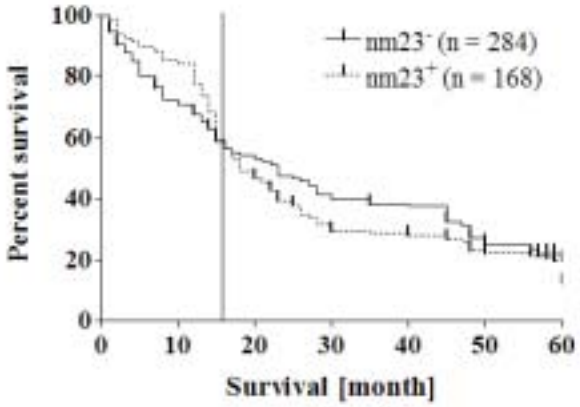
2. Expression of nm23-H1 was specific to tumor fraction (T) and metastatic lymph node (Ml) as compared to normal fraction (N) of the lung or the normal lymph node (Ln). Expression of nm23-H1 was determined by immunoblotting.



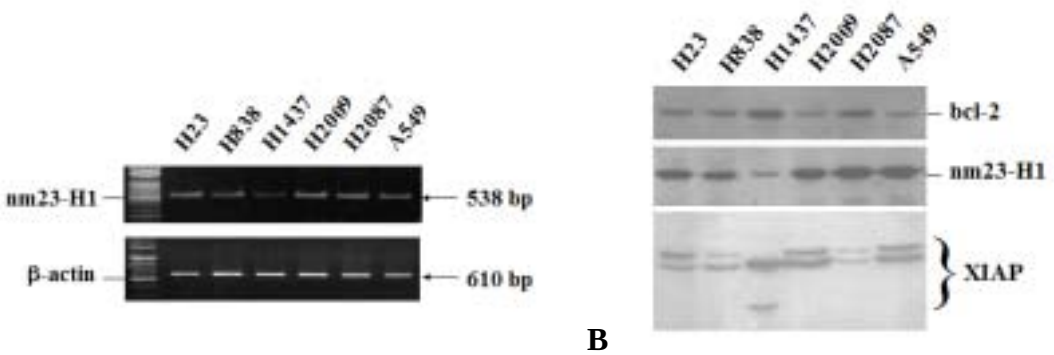
3. Representative of nm23-H1 expression as detected by immunohistochemistry. Expression of nm23-H1 was specific to (A) tumor cells and (B) metastatic lymph node



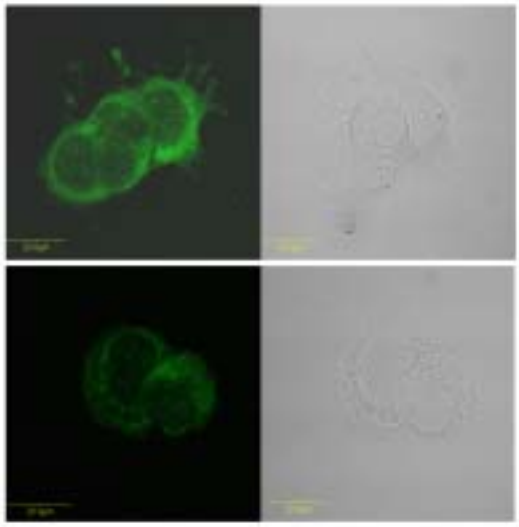
4. Although no significant difference was detected in survival analysis of nm23-H1 (P = 0.376), nm23-H1 curves, which were plotted by Kaplan-Meier method, did show peculiar skews and the two curves crossed with each other at 16-month. Interestingly, survival analysis of patient group with high nm23-H1 was worse in the earlier years, and then became better than that with low nm23-H1 after the second year. When data were re-grouped by using cross-point as a cut-off for each curve, the statistical differences were significant in both dissected regions (P = 0.037 and P = 0.021).



5. Screening of NSCLC cell lines that selectively expressing nm23-H1 by (A) RT-PCR, and (B) immunoblotting

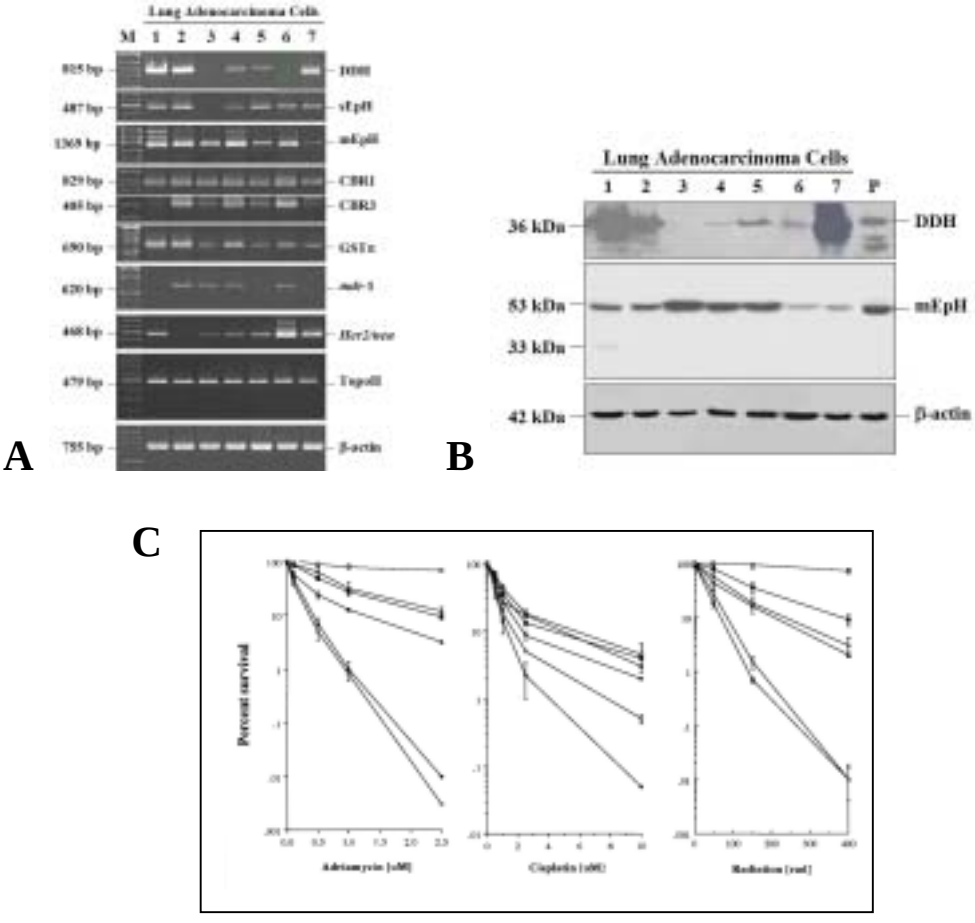


6. Differential expression and organelle localization of nm23-H1 as determined by confocal microscopy; upper panel: H23 and lower panel: H838

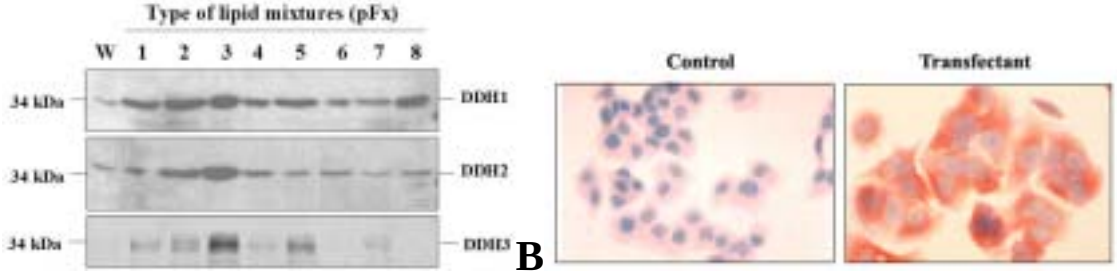


7. DDH overexpression induces drug- and radiation-resistance via suppression of DNA repair-associated gene expression in NSCLC

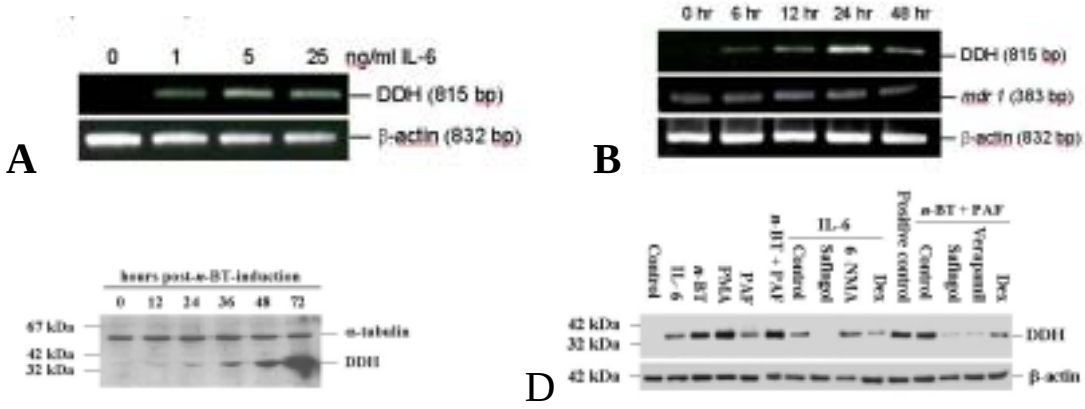
(1) DDH overexpression in NSCLC: Differential expression of DDH and mEpH in various lung cancer cell lines detected by RT-PCR (A) and immunoblotting (B); (C) the sensitivities in response to adriamycin, cisplatin and radiation treatments was measured by colony forming assay.



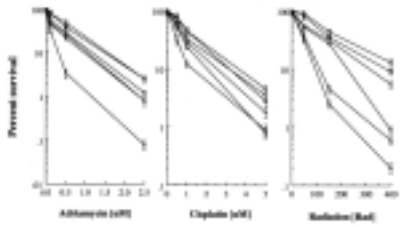
(2) Transfection of DDH gene increases DDH expression in lung adenocarcinoma cells: #6 cell that does not express DDH1 was transfected with edysone-inducible DDH 1 gene. (A) Efficacy of different transfectine mixtures; (B) Comparison of DDH expression before and after edysone-induction.



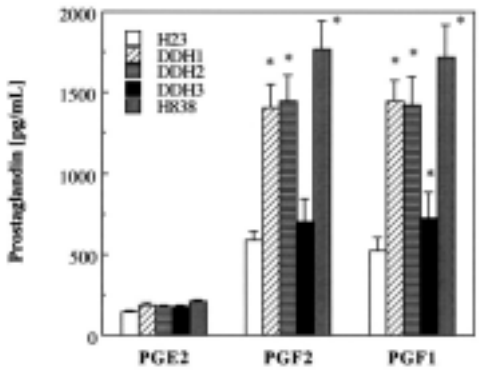
(3) Chemical induction of DDH expression: Expression of DDH1 could be induced by a variety of chemicals, such as IL-6, butyrate, phorbol ester and PAF. Induction effect could be inhibited by safingol and verapamil. (A) Dose-dependent induction of DDH expression by IL-6; (B) Time-dependent induction of DDH expression by IL-6; (C) Time-dependent induction of DDH expression by butyrate and (D) Chemical-induction and inhibition of DDH expression.



(4) Increase of DDH expression reduces drug- and radiation sensitivity in lung cancer cells: Induction of DDH expression by butyrate and its effect on the sensitivity to adriamycin and radiation treatments. Cytotoxicity was measured by colony forming assay.

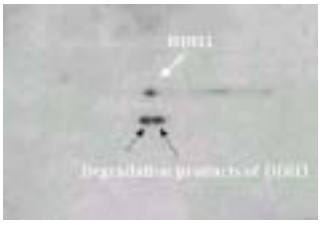


(5) Overexpression of DDH in lung cancer correlated with prostaglandin F synthesis



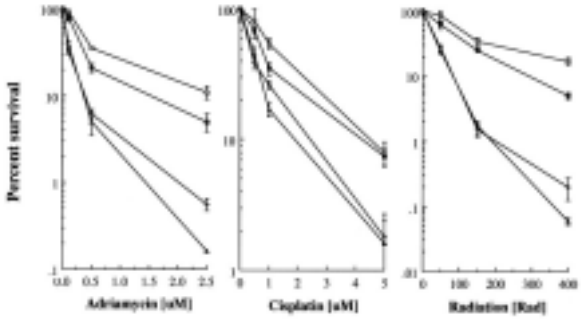
(6) Transfection of DDH and the confirmation of the transfected DDH gene product by a two-dimensional gel electrophoresis and Western blotting

DDH1/2 in mouse liver cell

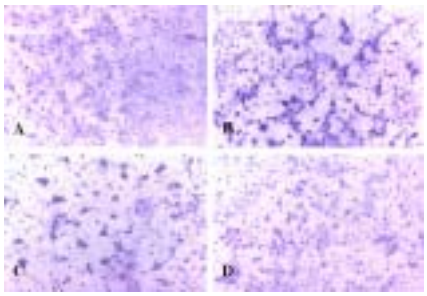


DDH1a & 1b in lung cancer cells

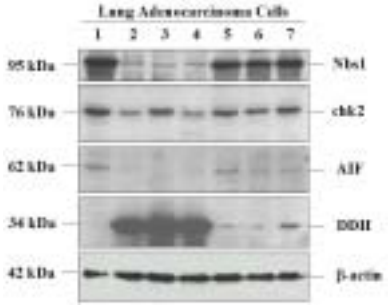
(7) Artificially introduced DDH expression reduces drug- and radiation sensitivity in lung cancer cells



(8) Higher DDH expression and the higher metastatic potential in lung cancer cells as measured by a matrigel penetration assay of umbilical endothelial cells

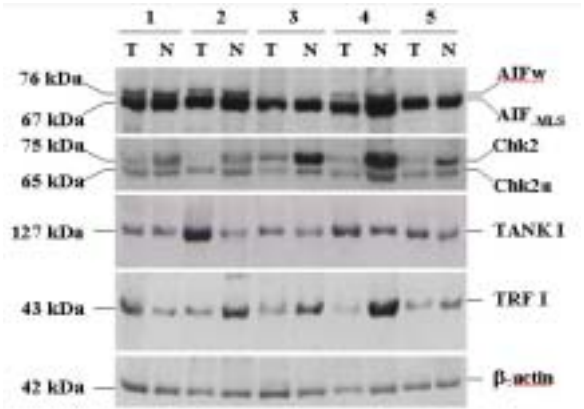


(9) Differential gene expression panel in lung cancer detected by western blot analysis



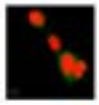
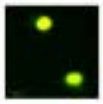
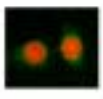
8. Different patterns of AIF expression and drug- and radiation-resistance in NSCLC

(1) Differential expression of DNA repair-related genes in tumor (T) and the normal tissues (N).

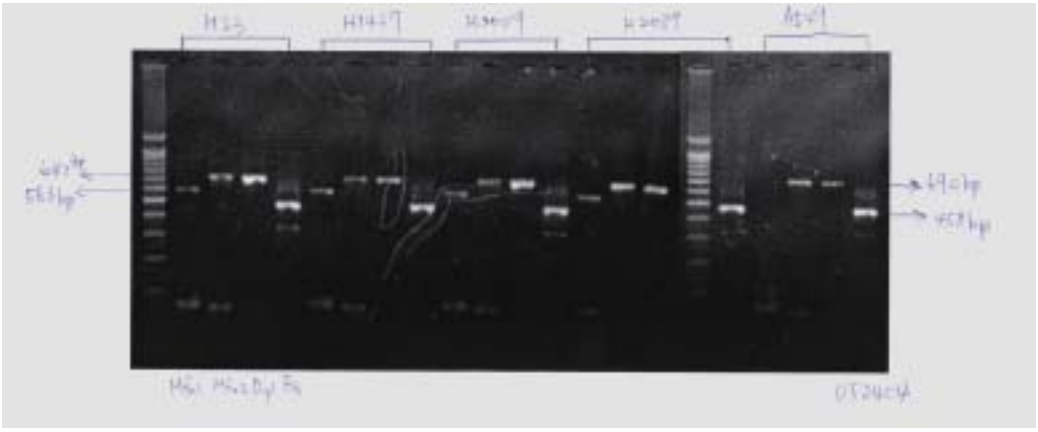


(2) Localization of AIF in the cancer cells by using confocal immunocytochemistry.

Localization of AIF was normally in the cytoplasm of cancer cells. AIF could be re-distributed into the nucleus following cisplatin treatment.

Cancer Cell line	Control	Radiation	Cisplatin
1			
2			
3			

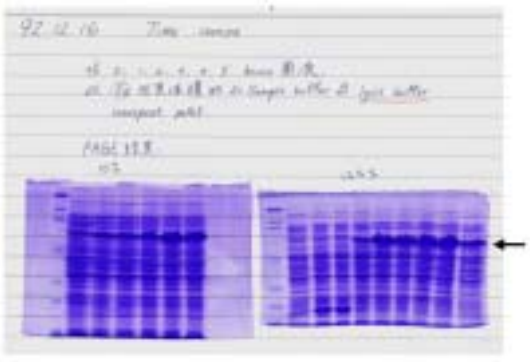
9. Differential expression patterns of mitochondria fusion (Mfn1 and Mfn2) and fission (Drp1 and Fis) genes in NSCLC; Mfn1 could be subjected to stimuli regulation in squamous type of lung cancer cells



10. Raising antibodies to mitofusin1/GTPase (Mfn1) (A) Subcloning of mitofusin1/GTPase gene into an expression vector, and to deliver recombinant DNA into bacteria to express recombinant mitofusin1/GTPases. (B) Western analysis of the expressed protein; (C) Purification of the expressed protein; (D) Western analysis of the purified expressed protein; (E) The preliminary screening of whole cell lysates from NSCLC with polyclonal antibodies to Mfn1. Arrow indicates the expressed Mfn1

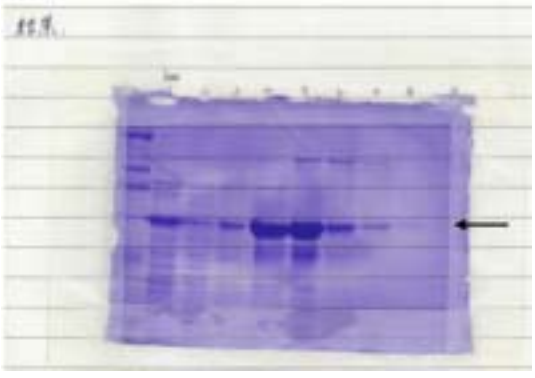
上一個進度報告我們認為由表現結果看來，蛋白質表現已無問題，然而其表現量偏低，需大量表現並進行純化進度及抗體之製備。因此我們改變表現蛋白的策略

A.

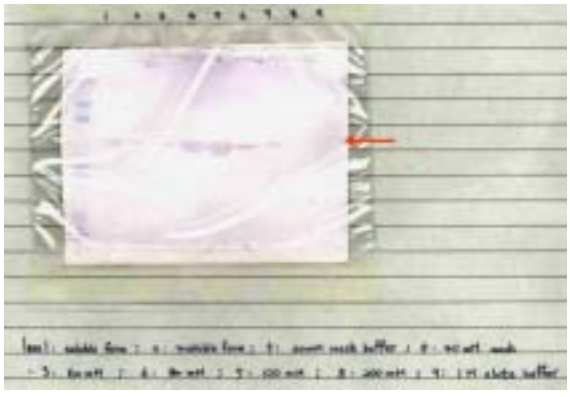


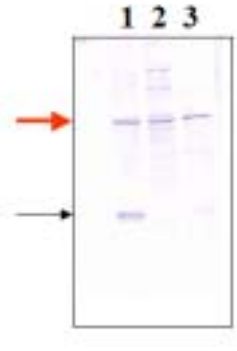
B.

C.



D.





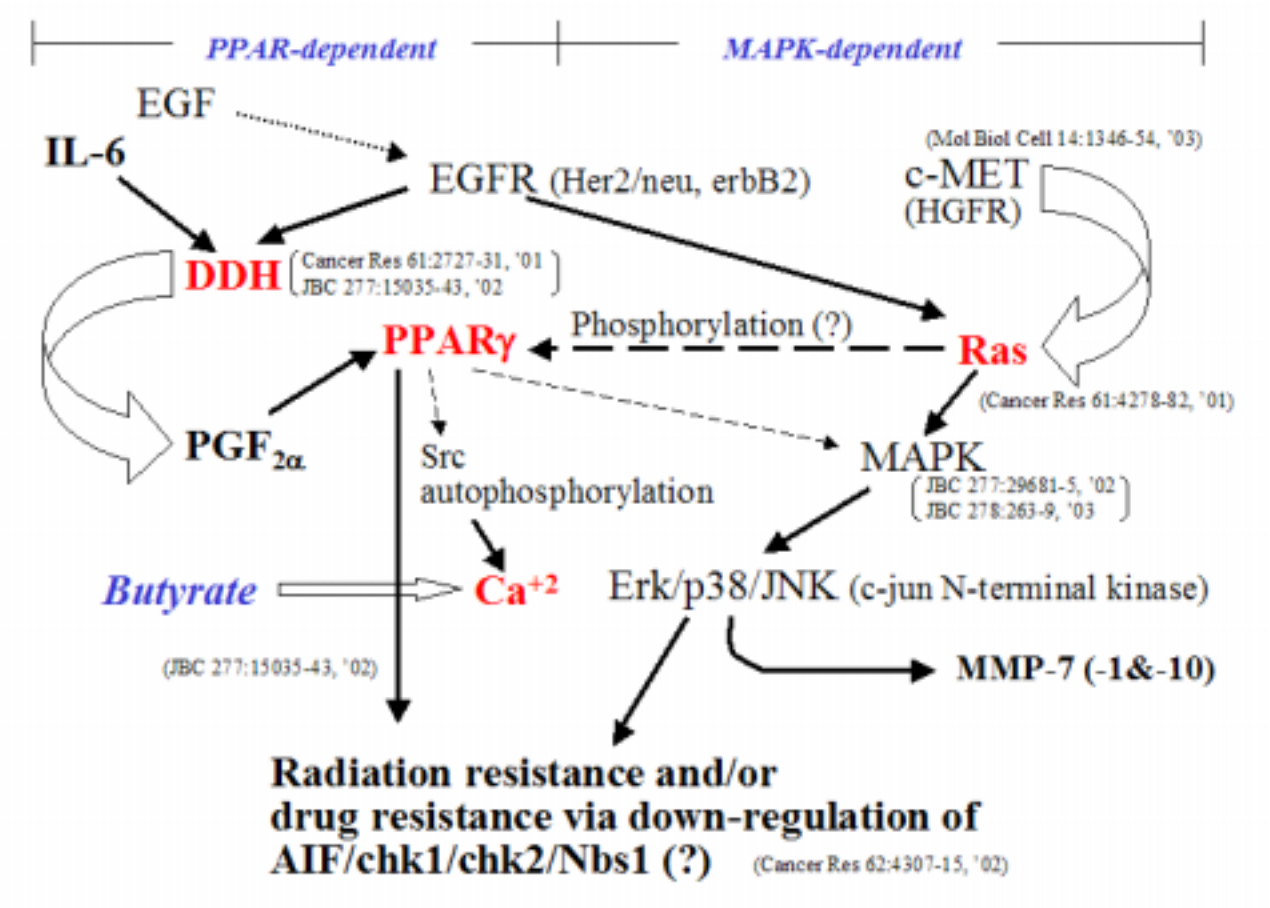
E.

From the results shown above, it is worth noting that, in a shotgun search, we have found that nm23 expression was inversely correlated with DDH, supported our anticipations that tumor progression is a concerted process, which includes a battery of genes expressing differentially at the different stages of disease. In particular, in patients with the advanced diseases, in whom the pathophysiological variables would be far more than a single gene could dominate, these variables involve IL-6, sIL-2Rα, DDH, COX-2 and nm23. The nm23-H1 expression was further shown associated with cisplatin-induced DNA damage. Although the detailed network of gene expressions is yet to be clarified, the effect of DDH and NM23 on drug resistance of cancer cells demands further investigation. In an ongoing study, the respective effects of the above mentioned variables are being evaluated.

As noted above, in addition to the involvement in cell proliferation, it is worth noting that in cisplatin-resistant ovarian carcinoma cells, Andrews *et al* detected that Na^+, K^+-ATPase expression was associated with the accumulation of cisplatin. Furthermore, nm23 gene transfection increased cisplatin sensitivity, and down-regulation of nm23-H1 prevented cisplatin-induced DNA damage implicate that nm23 could be directly associated with Na^+, K^+-ATPase. In fact, a multivariate analysis had obtained the similar result; in which nm23-H1 overexpression was most accountable for the increased survival of cancer patients received cisplatin-based chemotherapy. The *in vitro* abrogation of nm23-H1 expression by antisense RNA increased cisplatin resistance. Such results in fact fits pretty well with the recent finding of mitofucin (MTF) overexpression, a small mitochondria-associated G protein, in the drug-resistant cancer cell lines. Based on these data, that in addition to NDP kinase activity, nm23-H1 expression be related to Na^+, K^+-ATPase function, which in turn responsible for the decrease of intracellular accumulation of cisplatin and hence the drug resistance were then proposed. Moreover, cytotoxic effect of cisplatin was most potent during S to G2 phases, the period of cell cycle that nm23-H1 is preferentially expressed. It should be noted that if nm23-H1 positive cancer cells are more malignant, but more sensitive to cisplatin toxicity, then following ablative chemotherapy, the residual cancer cells, if there is any, would be potentially less malignant. Therefore, these patients can live with tumor of lesser vice and improve their survival. The correlation of AIF, and nm23 with DDH expressions, however, requires further clarification.

The connection could be easily associated by a signal, which provokes the full scheme gene activation. It is worth noting that the emerging evidences have suggested that in addition to growth factors, the process of tumorigenesis could be driven by the overexpression of COX-2, a ultimate enzyme in essence of inflammation. Biochemically, COX-2 overexpression is correlated with the expression and activation of HER family. Interestingly, it has been shown that lung cancers also express high levels of growth factors and their receptors, and lung cancer cell appear to exhibit autocrine- or paracrine-stimulated growth. Recently, four homologs of erbB tyrosine kinase receptors (type I receptor tyrosine kinases), erbB1/EGFR/HER1, erbB2/HER2/neu, erbB3/HER3 and erbB4/HER4, have been found. HER-2/neu proto-oncogene, which encodes a transmembrane glycoprotein with Mr. 185,000 ($p185^{neu}$), has been correlated with the disease progression of

human lung cancer. Since its first identification, HER-2/neu has been shown expressed in a wide variety of human malignancies, including carcinoma of the breast, salivary gland, ovary, gastrointestinal tract, liver, kidney and bladder. It was indicated that expression of HER-2/neu is associated with multidrug resistance, poor prognosis and short survival, and measurement of HER-2/neu should be considered as a marker for predicting the early recurrence and metastasis. An elegant observation by Gupta *et al* further added a very profound insight into this issue by demonstrating that ras pathway, a downstream substrate of EGFR and, possibly, erbB2, played a significant role in radiation resistance. Moreover, study of endosomal dynamics further suggest that EGFR and c-MET may have respective signal transduction pathways, though these two passages may cross talk [as shown in the following Figure]. In this study, we will determine if phosphorylation of PPARγ prior to DDH overexpression, which can transduce proliferation activation signal and, possibly drug- and radio-resistance via production of PGF2α, is a pre-requisite for the aberrant effect of Her2/neu, EGFR and/or c-Met. This has not been studied in detail, and it surely deserves a close look for it as a potential target of cancer therapy.



The putative networking of DDH expression with receptor kinase-associated gene activation and the related consequences of drug- as well as radio-resistance