行政院國家科學委員會專題研究計畫 成果報告

鳥胺酸去羧化酶對抗計畫性細胞死亡之機制
研究成果報告(精簡版)

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行政院國家科學委員會補助專題研究計畫■成果報告

（計畫名稱）

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中文摘要

鳥胺酸去羧化酶為多胺生物合成的關鍵酵素，在各種生物功能包括細胞增生、細胞死亡與細胞分化中扮演必要性角色。然而，鳥胺酸去羧化酶如何防止細胞凋亡的機制仍然未清楚瞭解。先前科學家研究已經證實鳥胺酸去羧化酶不可逆抑制劑difluoromethylornithine能夠降低其酵素活性，引起細胞內活性氧累積和細胞週期停滯，進而造成細胞死亡，這些發現顯示鳥胺酸去羧化酶可能具有抗氧化和抗細胞凋亡的作用。在本研究發現過度表現鳥胺酸去羧化酶具有類似活性氧清道夫的作用，能夠將腫瘤壞死因子-α (TNF-α)、methotrexate (MTX)和curcumin刺激後的細胞內活性氧減少、防止粒腺體膜電位瓦解與阻止細胞凋亡，而且它還能夠避免Bcl-2減少、阻止cytochrome c從粒腺體釋出與抑制劊蛋白酶-9跟劊蛋白酶-3的活性。根據這樣的結果，吾等建議鳥胺酸去羧化酶防止TNF-α、MTX和curcumin所誘發的細胞凋亡主要藉由抑制活性氧依賴性粒腺體調節的訊息途徑。此外，發現催乳激素(prolactin)以增加鳥胺酸去羧化酶活性，而提升Bcl-2表現，最後防止MTX所誘發細胞凋亡。在先前的文獻顯示Bcl-2為抗氧蛋白質，從吾等研究能夠瞭解鳥胺酸去羧化酶具有抗氧化作用，其中之一的機制與Bcl-2的作用有著密切相關性。

成果報告 壹

Abstract

Prolactin has more than 300 separate functions including affecting mammary growth, differentiation, secretion and anti-apoptosis. In the previous studies, prolactin induced Bcl-2 expression to prevent apoptosis and also provoked the activity of ornithine decarboxylase (ODC). Our previous data showed that ODC overexpression upregulates Bcl-2 and prevents tumor necrosis factor alpha (TNF-α)- and methotrexate (MTX)-induced apoptosis. Here, we further investigate whether prolactin prevents MTX-induced apoptosis through inducing ODC activity and the relationship between ODC and Bcl-2 upon prolactin stimulation. Prolactin prevented MTX-induced apoptosis in a dose-dependent manner in HL-60 cells. Following prolactin stimulation, ODC enzyme activity also shows an increase in a dose-dependent manner, expressing its maximum level at 3 h, and rapidly declining thereafter. Prolactin-induced ODC activity is completely blocked by a protein kinase C delta (PKCδ) inhibitor, rottlerin. However, there are no changes in the expressions of ODC mRNA and protein level after prolactin stimulus. It indicates that prolactin may induce ODC activity through the PCKδ pathway. Besides, Bcl-2 expresses within 1 h of prolactin treatment and this initiating effect of prolactin is not inhibited by alpha-difluoromethylornithine (DFMO). However, Bcl-2 is further enhanced following prolactin stimulation for 4 h and this enhancement is blocked by DFMO. Bcl-2 has no effect on ODC activity and protein levels, but ODC upregulates Bcl-2, which is inhibited by DFMO. Overall, there are two different forms of prolactin effect, it induces Bcl-2 primarily, and following this it stimulates ODC activity. Consequently induced ODC activity further enhances the expression of Bcl-2. The anti-apoptotic effect of prolactin is diminished by DFMO and recovered by putrescine. Obviously, ODC activity is one basis for the anti-apoptotic mechanisms of prolactin. A Bcl-2 inhibitor, HA14-1, together with DFMO, completely blocks the anti-apoptotic effects of prolactin. These results suggest that increasing ODC activity is another way of prolactin preventing MTX-induced apoptosis and that this induction of ODC activity enhances the expression of Bcl-2 strongly enough to bring about the anti-apoptotic function.

Introduction

Prolactin is a versatile hormone, produced in the anterior pituitary gland. Its major function is to affect the growth, differentiation and secretion of the mammary glands. In addition, it has more than three hundred separate functions, including regulating cellular function, such as proliferation, differentiation, angiogenesis and protection against apoptosis and inflammation, as a cytokine.[1, 2] Prolactin functions as a potent survival factor for human breast cancer cells,[3] PC3 prostate cancer cells,[4] human granulosa cells,[5] thymocytes[6] and Nb2 lymphoma cells.[7] The known mechanisms of this anti-apoptotic function are consistent with prolactin-induced upregulation of Bcl-2,[8, 9] Bcl-xL,[10] or downregulation of Bax and augmentation of Bcl-2/Bax ratio.[11]

Several genes associated with cell growth and apoptosis, including c-*myc* and ornithine decarboxylase (ODC; EC 4.1.1.17), are rapidly induced within four hours of prolactin addition to the Nb2 lymphoma cell line.[12] Prolactin induces the activity of ODC and the expression of its mRNA in a variety of cell lines or tissues.[13-16] In a previous study, it was demonstrated that spermine, one of the polyamines, could block DNA fragmentation in dexamethasone-induced apoptosis. However, an ODC irreversible inhibitor, alpha-difluoromethylornithine (DFMO), did not inhibit the anti-apoptotic effect of prolactin.[17] This demonstrates that there are multifarious regulations of survival factors involving the anti-apoptotic effect of prolactin.

ODC, the first and rate-limiting enzyme of the polyamine biosynthetic pathway, decarboxylates $_{L}$-ornithine to form putrescine.[18] ODC and polyamines (putrescine, spermidine and spermine) play an important role in several biological functions, including embryonic development, the cell cycle and proliferation,[19] and the origin progression of neoplastic diseases.[20, 21] Our previous studies showed overexpression of ODC prevented tumor necrosis factor-α (TNF-α)- and methotrexate (MTX)-induced apoptosis.[22, 23]

MTX, the 4-amino, 10-methyl analogue of folic acid, is the most widely used anti-folate in cancer chemotherapy and in the treatment of nonmalignant disorders such as psoriasis, rheumatoid arthritis and graft-versus-host diseases.[24] Low doses of MTX induce apoptosis of mitogen-activated $CD4^+$ and $CD8^+$ T cells, but not resting T cells. They also lead to clonal deletion of activated T cells in mixed lymphocyte reactions.[25, 26] We have demonstrated that low doses of MTX induce apoptosis via reactive oxygen species (ROS)-dependent and the mitochondria-mediated pathway, and overexpression of ODC prevents MTX-induced apoptosis. ODC overexpression can reduce ROS, increase Bcl-2 expression, stabilize mitochondrial membrane potential, inhibit cytochrome c release and prevent caspase activation. Here, we further explore whether prolactin can prevent MTX-induced apoptosis through inducing ODC activity and the relationship between ODC and Bcl-2 upon prolactin treatment.

Materials and methods

Cell culture and chemical materials

Human promyelocytic leukemia HL-60 cells were grown in 90 % RPMI 1640 and 10 % fetal bovine serum (FBS) obtained from Gibco BRL (Grand Island, NY) at a temperature of 37°C in a humidified, 5 % CO_2 atmosphere. MTX, ribonuclease A (RNase A), putrescine, acridine orange, propidium iodide (PI), HA14-1 and prolactin were purchased from Sigma (St Louis, MO), and DFMO was obtained from Calbiochem (La Jolla, CA).

Cell viability and acridine orange staining
Cell numbers were counted using trypan blue exclusion assay. The extent of cell viability was calculated by the viable cell numbers from experiment groups in contrast with the untreated control group. To identify apoptotic character upon MTX stimulation, 5 x 10^4 cells in 10 µl cell suspension were mixed with equal volumes of acridine orange solution (10 µg/ml) in phosphate-buffered saline (PBS) on each slide. Green fluorescence was detected by microscope as being between 500-525 nm (Olympus America, St Huntington, NY).[27]

DNA fragmentation analysis
Cells (5 x 10^6) were harvested in PBS and lysed overnight in a digestion buffer (0.5 % sarkosyl, 0.5 mg/ml proteinase K, 50 mM Tris-HCl, pH 8.0 and 10 mM EDTA) at 55°C. Subsequently, cells were treated with 0.5 µg/ml RNase A for 2 h. The genomic DNA was extracted by phenol-chloroform-isoamyl alcohol and analyzed by gel electrophoresis at 50 volts for 90 min using 2% agarose. Approximately 20 µg of genomic DNA was loaded in each well, visualized under ultraviolet (UV) light and photographed.

Apoptotic sub-G1 analysis
1 x 10^6 cells were cultured in 35-mm petri dishes and incubated for 24 h. Cells were pretreated with prolactin and/or treated with drugs for the specified time and dose, then harvested, washed with PBS, resuspended in 0.2 ml of PBS and fixed in 0.8 ml of ice-cold 100% ethanol at -20°C overnight. The cell pellets were collected by centrifugation, resuspended in 1 ml of hypotonic buffer (0.5% Triton X-100 in PBS and 0.5 µg/ml RNase A) and incubated at 37°C for 30 min. Then, 1 ml of PI solution (50 µg/ml) was added and the mixture was allowed to stand on ice for 30 min. The nuclei were analyzed in a FACSCAN laser flow cytometer (Becton Dickenson, San Jose, CA).

Human *odc* gene sub-cloning and cell transfection
Parental HL-60 cells were grown in RPMI 1640 medium supplemented with 10 % heat-inactivated FBS for 3 h, then the harvested cells were gently rinsed in PBS. Purification of mRNA was carried out according to the supplier's instructions (MDBio, Taiwan) and the cDNA was synthesized by reverse transcriptase (RT) (Promega, Madison, WI). Polymerase chain reaction (PCR) amplification of the encoding region of the human *odc* cDNA was performed with our designed primers derived from the human *odc* sequence. The PCR product was sub-cloned to a eukaryotic expression vector, pCMV-Tag (Novagen, Madison, WI) and then sequenced. The plasmid of ODC expression was constructed by inserting the *Bam*HI-*Eco*RI 1,415 bp coding region fragment. Parental HL-60 cells were transfected with WT-ODC (overexpressing ODC) and m-ODC (frame-shift mutant ODC) plasmids according to calcium phosphate-mediated transfections, respectively.[28] Stably transfected cells were selected with the antibiotic G418 (400 µg/ml). Three weeks later, isolated G418-resistant clones were individually analyzed for expression of ornithine decarboxylase. The ODC expressions of individual clones were examined by RT-PCR, immunoblotting and enzyme activity assay. Overexpressed Bcl-2 cells were built as previously described.[29]

ODC enzyme activity assay
ODC enzyme activity was assayed at 37°C by measuring its product, putrescine, as described previously[30] with the following modification. Samples were suspended in ODC buffer (50 µM EDTA, 25 µM pyridoxal phosphate and 2.5 mM DTT in 25 mM Tris-HCl, pH 7.1) incubated with 2 nmole of L-ornithine for 1 h, and then the material was spotted onto p81 phosphocellulose (Whatman, Maidstone, England). Diamines were eluted from the dried papers by shaking at 37°C for 60 min with 0.5 ml quantities of elution buffer (0.5 M magnesium chloride in 0.2 M boric acid–borax buffer, pH 8.4). Following that, samples were supplemented with 400 µl of luminescence reagent [11.7 µg/ml luminal, 30 µg/ml peroxidase type II (EC 1.11.1.7) and 67 mM glycine buffer, pH 8.6 (at 1:1:2.5, v/v/v)] for each cuvette. While keeping the cuvettes in the dark for 30 min, the background was measured in the TR 717 microplate luminometer (Perkin-Elmer, Foster, CA). Then 5 µl of diamine oxidase (4.61 µg/µl) (Sigma) were injected into each cuvette. Luminescence was recorded for 40 sec at 37°C and results were calculated according to the standard curve using putrescine.

RT-PCR
RNA was isolated from cells by Trizol (MDBio) according to the manufacturer's instructions. Synthesis of cDNA was performed using mRNA templates, RT and 500 ng of random primers. The reaction mixture was incubated for 90 min at 42°C. For the PCR assay, cDNA was added to 40 µl mixture buffer containing 75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20 (v/v), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM forward and reverse primers and 1 U Taq DNA polymerase (MDBio). PCR was set on the condition of 2 min at 94°C, 25–35 cycles (30 s, 94°C; 30 s, 56–60°C; 15–30 s, 72°C) and 10 min at 72°C by a Mastercycler (Eppendorf). The products were analyzed on 1.5% agarose gel. The following primer pairs were used: β-actin (309 bps) 5′-AGCGGGAAATCGTGCGTG-3′ and 5′-CAGGGTACATGGTGGTGC-3′; ODC (533 bps) 5′-TTACTGCCAAGGACATTCTG-3′ and 5′-GCTGACACCAACAACATCG-3′.

Immunoblotting
To purify all of the proteins, cells were harvested, lysed in cold lysis buffer (10% v/v glycerol, 1% v/v Triton X-100, 1 mM sodium orthovanadate, 1 mM EGTA, 10 mM NaF, 1 mM sodium pyrophosphate, 20 mM Tris, pH 7.9, 100 µM β-glycerophosphate, 137 mM NaCl, 5 mM EDTA, 1 mM PMSF, 10 µg/ml aprotinin and 10 µg/ml leupoptin), homogenized and centrifuged. The supernatant was boiled in loading buffer and an aliquot corresponding to 100 µg of protein separated by SDS-PAGE was used. Following blotting, the membranes were incubated with anti-ODC (MDBio), anti-Bcl-2, anti-Bcl-xL, anti-α-tubulin antibodies (Santa Cruz, Santa Cruz, CA) for 6 h and the second antibody labeled with horseradish-peroxidase was adjacently incubated for 1 h. The antigen-antibody complexes were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). The expression of protein levels was quantified with LAS-1000plus density meter (Fujifilm, USA).

Statistical analysis
Statistical analysis for significant differences between the control and experimental groups were evaluated using Student's *t* test.

Results

Prolactin prevents MTX-induced apoptosis in a dose-dependent manner

We have previously demonstrated that MTX induces apoptosis via a ROS-dependent and mitochondria-mediated pathway.[23] Here, we focus on whether prolactin has an anti-apoptotic effect on MTX-induced apoptosis. HL-60 cells were pretreated with 0, 0.1, 1 and 5 ng/ml of prolactin for 3 h and then treated with 1 µM MTX. Cells were observed by

fluorescence microscope and the surviving cells were counted to ascertain overall cell viability. MTX induced cell death and caused the decrease of cell viability to 54±5% at 24 h when compared with control cells. Following cells pretreated with prolactin for 3 h, cell viability was increased in a dose-dependent manner when compared with cells treated by MTX alone. Prolactin (5 ng/ml) increased the extent of cell viability up to 108±4% at 24 h, that is, it promoted cell proliferation (Fig. 1A). The typical apoptotic morphologic changes induced by MTX, such as chromatin condensation, membrane blebbing, shrinkage and apoptotic body formation, were blocked by 5 ng/ml prolactin (Fig. 1B). Following incubation of 1 μM MTX for 24 h with or without pretreatment of the different doses of prolactin, the genomic DNA was extracted from harvested cells and analyzed by DNA gel electrophoresis. The results show that there is a dose-dependent effect of prolactin on decreasing DNA fragmentation after MTX treatment (Fig. 1C). The sub-G1 ratio, assayed by flow cytometry with PI staining, showed a significantly greater decrease in the cells pretreated with 5 ng/ml prolactin (sub-G1 ratio: 8.3%) than those without pretreatment (sub-G1 ratio: 40.4%) (Fig. 1D). The result confirmed that the effect of decreasing sub-G1 ratio by pretreatment of 5 ng/ml prolactin for 3 h was more efficient than by pretreatment of 1 mM putrescine (22.6%) or ROS scavengers, such as 10 mM *N*-acetylcysteine (25.3%) for 3 h, 1 mM vitamin C (20.8%) or 100 U catalase (20.6%) for 1 h, respectively.[23]

Prolactin induces ODC activity in a dose-dependent manner

Prolactin increases the expression and activity of ODC in transcriptional, and possibly posttranslational, levels in various cell lines.[12] Only one report has demonstrated that prolactin induces ODC activity in HL-60 cells.[13] We treated HL-60 cells with 0, 1, 1.75, 2.5, 5 and 10 ng/ml prolactin for different times. The cells were then harvested to measure the activity, mRNA and protein of ODC. In the time-course experiment, ODC activity was increased to the maximum after 3 h of prolactin stimulation at the quantity of 2.5 and 5 ng/ml (Fig. 2A). Rottlerin, a relative selective PKCδ inhibitor, blocked the effect of 5 ng/ml prolactin on ODC activity within cells at 2 μM (Fig. 2A), however rottlerin did not inhibit ODC enzyme activity *in vitro* (data not shown). Simultaneously, there is a dose-dependent effect of prolactin on inducing ODC activity at 3 h (Fig. 2B). Although prolactin increased ODC activity, there were no changes in ODC mRNA (Fig. 2C) and protein expression (Fig. 3A and 3B) after different times and dosage levels of treatment. Our data reveals that prolactin induces ODC activity in a dose-dependent manner, which might be directly or indirectly affected by PKCδ.

Prolactin upregulates Bcl-2, but not Bcl-xL in HL-60 cells

There are several studies that demonstrated prolactin up regulates Bcl-2 or Bcl-xL to prevent apoptosis in Nb2-T cell line from many insults.[8-10] Here, HL-60 cells were treated with 5 ng/ml prolactin for different time periods. All of the protein was extracted for immunoblotting with anti-Bcl-2, anti-Bcl-xL antibodies. Bcl-2 is upregulated within one hour of prolactin treatment, and further enhanced later in HL-60 cells, but the Bcl-xL level does not increase (Fig. 3A and 3B).

Bcl-2 has no effect on the activity and expression of ODC

Prolactin enhanced both Bcl-2 and the activity of ODC in previous studies. However, there is less known association between Bcl-2 and ODC. We first examined whether Bcl-2 affects ODC or not. Our data revealed prolactin upregulates Bcl-2 (1 h after treatment) before the activity of ODC becomes active (3 h after treatment). We transfected the *bcl-2* gene or its vector only into parental HL-60 cells, respectively. HA14-1 is a cell permeable and small non-peptidic organic ligand of Bcl-2, which is found to bind Bcl-2 with the IC_{50} of 9 μM in competing with the Bcl-2 binding of Flu-BakBH3.[31] HA14-1 induces apoptosis of HL-60 cells that is associated with the decrease in mitochondrial membrane potential and activation of caspase 9. When HL-60 cells was treated with 5 μM HA14-1, we found that HA14-1 decreased the expression of Bcl-2 and increased the loss of mitochondrial membrane potential, however, it did not cause cell death in 24 h (data not shown). After being stimulated by 10% FBS with or without 5 μM HA14-1, parental HL-60 cells and Bcl-2 cells were harvested to measure the activity of ODC and protein levels by enzyme assay and immunoblotting for the indicated time. The results show that for overexpression of Bcl-2 or treatment of HA14-1 neither affects the activity (Fig. 4A) nor protein expression (Fig. 4B) of ODC in any time of stimulus.

ODC could upregulate Bcl-2

In our previous studies, overexpression of ODC enhanced the expression of Bcl-2 and prevented the decline of Bcl-2 following TNF-α and MTX treatment.[22, 23] We constructed ODC cDNA into a mammalian expression plasmid, pCMV-Tag and generated cell line overexpressing ODC in parental HL-60 cells, termed by WT-ODC. Parental HL-60 cells were also transfected by its frame-shift mutant vector as control, termed by m-ODC. In our previous studies, ODC activity after serum induction was completely blocked by 1 mM DFMO, however, it did not cause cell death.[22, 23] After being stimulated by 10% FBS with or without 1 mM DFMO for 3 h, all of the proteins from the WT-ODC and m-ODC cells were harvested and Bcl-2 was detected by immunoblotting. The results showed that DFMO decreased the expression of Bcl-2 by up to 70% in m-ODC cells. WT-ODC cells had 2.8-fold increases of Bcl-2 compared with m-ODC cells. The effect of overexpressed ODC-induced Bcl-2 protein levels was further lessened by DFMO (Fig. 5). That result demonstrates that ODC has an up-regulatory effect on Bcl-2 expression.

Prolactin prevents MTX-induced apoptosis via upregulation of both ODC activity and Bcl-2

It was been demonstrated in our previous study that overexpression of ODC or Bcl-2 prevents MTX-induced apoptosis.[23] We further investigated whether induction of ODC activity is a mechanism by which prolactin prevents MTX-induced apoptosis. HL-60 cells were pretreated with 5 ng/ml prolactin combined with or without 1 mM DFMO for 3 h, then treated by with 1 μM MTX, 5 μM HA14-1 or 1 mM putrescine. Cell survival and death were observed by fluorescence microscope, and sub-G1 group for the indicated time detected by flow cytometer. DFMO alone did not cause cell death in HL-60 cells. The percentages of cell viability in the groups treated by prolactin and MTX, or by prolactin, MTX, DFMO and putrescine were significantly larger than the groups treated by MTX alone or by prolactin, MTX and DFMO (Fig. 6A). The results of apoptotic cells (sub-G1) ratio in various experiments were similar to the decreases in the percentages of cell viability (data not shown). These results show that DFMO has an inhibitory effect on the protective function of prolactin, which is improved by putrescine. Prolactin-induced ODC activity has a role in preventing MTX-induced apoptosis. Next we used HA14-1, the Bcl-2 ligand, to block Bcl-2 function. When only treated with 5 μM HA14-1 in HL-60 cells, it doesn't induce cell death

within 24 h (data not shown). However cells were pretreated with 5 ng/ml prolactin and 1 mM DFMO, then treated by MTX with or without HA14-1. HA14-1 caused a significant in decrease cell viability at 6 h when compared with no HA14-1 addition ($p<0.05$), but this decrease in cell viability was not apparent at 24 h ($p=0.6287$) (Fig. 6A). It is shown that prolactin-induced Bcl-2 affords a protective effect during the early stage of this condition without ODC activity. To confirm again that this protective effect was due to Bcl-2 induced by prolactin, we compared cell viability between HL-60 cells treated with 1 μM MTX alone and cells that were pretreated by 5 ng/ml prolactin for 3 h and then treated with 1 μM MTX or 1 μM MTX combined with 5 μM HA14-1. These results were compatible with the previous findings in Fig. 6A and demonstrated that the anti-apoptotic effect of prolactin in the first 6 hours was completely blocked by 5 μM HA14-1. However prolactin still had some anti-apoptotic effect at 12 h and 24 h (Fig. 6B). HA14-1 combined with DMFO completely blocked the anti-apoptotic effect of prolactin. The results indicate that prolactin's rescue of HL-60 cells from MTX insults is aided by both ODC activity and Bcl-2.

Prolactin-induced ODC activity is not required to upregulate Bcl-2 early, but indispensable in enhancing it later

To clarify the changes and relationships of ODC activity and Bcl-2 following prolactin stimulation, HL-60 cells were treated with or without 5 ng/ml prolactin and 1 mM DFMO. Following cells being harvested at 0, 1 and 4 h, Bcl-2 and α-tubulin were detected by immunoblotting, respectively. Prolactin induced 1.4-fold increases in Bcl-2 in the first hour, and further enhanced 2-fold of Bcl-2 expression at 4 h when compared with the initial time. The increase of Bcl-2 in the first hour was not inhibited by DFMO, however the effect of enhancing its expression was blocked by DFMO for a 0.7-fold decrease at a late stage (Fig. 7). The results indicate that prolactin-induced Bcl-2 expression, in the beginning at least, is independent of ODC activity. However, prolactin-induced ODC activity reaches a peak 3 h following treatment (Fig. 2A) and then enhances Bcl-2 expression 4 h later (Fig. 7).

Discussion

Prolactin prevents apoptosis from several insults, including C2-ceramide,[3, 5] TRAIL,[4] dexamethasone[6] and nitric oxide.[7] Here, we provide further evidence to prove that prolactin can prevent MTX-induced apoptosis. The time-course and dose-dependent studies of cell viability and DNA gel electrophoresis showed prolactin increases cell viability and decreases DNA fragmentation. The sub-G1 ratio by flow cytometry was significantly decreased by pretreatment with 5 ng/ml prolactin. The ability of the prolactin-decreasing sub-G1 ratio is better than putrescine, overexpression of ODC and ROS scavengers, such as *N*-acetylcysteine, vitamin C and catalase, respectively. It appears that prolactin supplies more powerful survival factors than putrescine, ODC and ROS scavengers.

After being stimulated by prolactin by a variety of doses, we found ODC activity is dose-dependently increased up to maximum at 3 h followed by a rapid decline in HL-60 cells. This indicates that prolactin induce ODC activity in a dose-dependent manner in the first three hours. However, there was no change in the expression of ODC mRNA and protein. The results support the contention that prolactin-induced ODC activity might occur though a posttranslational regulation in HL-60 cells. ODC activity is transiently induced by various exogenous stimuli.[32] It was observed that increases in ODC mRNA or protein are usually much less or occur later than its activity,[33-35] indicating that posttranslational regulation play a role in the induction of ODC activity. There are multiple forms of ODC found in various tissues and cells by isoelectric focusing analyses.[36-39] Furthermore, it has been demonstrated that ODC is phosphorylated *in situ* at serine and threonine residues.[39] Phosphorylated ODC is more stable and has 50% greater catalytic efficiency than unphosphorylated forms in RAW264 cells.[40] The protein kinase known to phosphorylate ODC is casein kinase II (CKII).[41] A consensus sequence for CKII-catalyzed phosphorylation is conserved in ODC amino acid sequence around serine 303. Neither mutation of ODC serine 303[42-44] to alanine had an effect on ODC activity,[45] nor did phosphorylation of ODC by CKII.[40, 46, 47] In addition, serine is the only ODC amino acid residue modified by CKII *in vitro*.[45] In RAW 264 cells, there are phosphothreonine residues found in phosphorylated ODC, that is, *in situ* phosphorylation of ODC threonine residues is catalyzed by an unidentified protein kinase(s) other than CKII.[40] The activity of CKII in rat lymphoid Nb2 cells was 2-fold higher than in the control 24 h later following prolactin treatment.[48] The slow stimulation of CKII by prolactin in Nb2 cells suggests that this kinase is not specifically induced and is not responsible for inducing ODC activity in prolactin action. Our results demonstrate that prolactin rapidly induces ODC activity but doesn't affect ODC mRNA and protein expression. We speculate that ODC is modified posttranslationally to increase the activity. CKII does not seem the key enzyme in posttranslational regulation of ODC after prolactin treatment.

Among the intracellular signals triggered by PKCs are ones of the known pathways that induce ODC activity by interleukin-2,[49] prolactin[50] and 12-O-tetradecanoyl-phorbol-13-acetate (TPA).[51] In human hepatoma HepG2 cells, hepatocyte growth factor (HGF) induces ODC activity in 4 h. However, expression of ODC mRNA occurs in 8-10 h.[35] The induction of ODC activity by HGF is blocked by different protein kinase inhibitors, including a PKC inhibitor, H7.[35] ODC activity induced by HGF seemed to be regulated posttranscriptionally and PKC might be involved in this regulatory process. In the studies of PKCδ transgenic mice, there is a significantly greater increase in TPA-induced epidermal ODC activity than their wild-type littermates.[52, 53] In cells overexpressing mutant PKCδ, the PKCδ activity and induction of ODC mRNA (3-fold), protein (7-fold) and activity (12-fold) are all completely inhibited in response to H_2O_2. H_2O_2 upregulates ODC expression transcriptionally and possibly posttranscriptionally via PKCδ.[34] However, fold-increases in ODC mRNA and protein are much less than the increase in activity, suggesting that ODC may also undergo posttranscriptional regulation in the presence of oxidants, which PKCδ has an effect on. PKCδ was observed to be the key component of the TPA and oxidative stress induced signal transduction pathways for the induction of ODC activity. In our experiments, rottlerin, a relative selective PCKδ inhibitor, completely blocks ODC activity induced by prolactin. PKCδ might play a role in directly or indirectly regulating the activation of ODC.

ODC is tightly regulated and has a very short half-life in many different cells.[32] It is known that antizyme is central in the regulation of ODC. Antizyme has higher affinity for the ODC monomer and blocks the formation of enzymatically active ODC homodimer. It also acts catalytically to direct the proteasome to degrade ODC protein. Antizyme production depends on polyamine levels through an unusual mechanism, one that uses translational frameshifting.[54] Our results showed ODC activity is increased by prolactin, but there are no changes in the expression of protein and mRNA. We can speculate that ODC activity might be largely upregulated by phosphorylation(s).

Phosphorylated ODC is more stable, however unphosphorylated and newly produced ODCs are degraded more rapidly due to increased antizyme through prolactin induced ODC activity. This is the possible reason for keeping the balanced amount of ODC protein with a higher enzymatic activity. In addition, the question of whether prolactin treatment might alter the association between ODC and antizyme will be further investigated.

In our study, HL-60 cells express more Bcl-2 protein as early as one hour after being stimulated by prolactin, but not Bcl-xL. We will now further clarify the relationship between Bcl-2 and ODC. Bcl-2 overexpression or inhibition by HA14-1 cannot affect the activity and protein expression of ODC, which indicates that Bcl-2 has no effect on ODC. A previous study demonstrated that DFMO could lower Bcl-2 protein content and putrescine could raise its expression.[55] Moreover, our data show that DFMO decreases Bcl-2 expression and overexpression of ODC increases its term. ODC activity achieves the upregulation of Bcl-2. Prolactin-induced Bcl-2 occurs within one hour earlier than increasing ODC activity and Bcl-2 expression isn't inhibited by DFMO in this early stage. Appreciably, the mechanism of prolactin-induced Bcl-2 in the early stage does not occur through ODC activity. Nevertheless, Bcl-2 expresses at 4 h after prolactin stimulation and which is reduced by DFMO. Altogether, prolactin first induces Bcl-2 and then enhances ODC activity. There is no association between Bcl-2 and ODC activity in the initiation of prolactin treatment. Although there is no effect of ODC on the early stage of prolactin-induced Bcl-2 expression, provoked ODC activity further increases Bcl-2 at a late stage. In addition, overexpression of ODC prevents Bcl-2 decline upon TNF-α and MTX treatment.[22, 23]

The inhibition of ODC activity by DFMO diminishes the protective effect of prolactin on MTX-induced apoptosis. There is no significant anti-apoptotic effect at 24 h under the situation that prolactin-induced ODC activity is blocked. Exogenous putrescine overturns the inhibitory effect of DFMO on prolactin action. Furthermore Bcl-2 has no effect on ODC. These results demonstrate that induced ODC activity does not rely on Bcl-2 expression during the progression of prolactin preventing MTX-induced apoptosis.

Upregulation of the Bcl-2 expression by prolactin is observed in several studies, and thought to be associated with the anti-apoptotic action of prolactin.[9, 56] However, simultaneous addition of prolactin and glucocorticoid in Nb2 cells fails to maintain even normal levels of this anti-apoptotic protein.[57] Therefore, there is a question as to the role of Bcl-2 in the prolactin-protected apoptotic effect. In our studies, overexpression of Bcl-2 prevents MTX-induced apoptosis.[23] Prolactin induces Bcl-2 expression in the condition of blocked ODC activity by DFMO. When cells were pretreated with prolactin and DFMO, there is still a protective effect on MTX-induced apoptosis. HA14-1 significantly enhances cell death at 6 h, but not at 24 h. That is, Bcl-2 induced by prolactin in the early stage affords an initial protective effect. Without the effects of ODC activity, prolactin can't prevent apoptosis 24 h later. It has been illustrated that prolactin-induced ODC activity is important in anti-apoptotic action. DFMO combined with HA14-1 completely blocks the anti-apoptotic effects of prolactin in HL-60 cells. This suggests that ODC activity and Bcl-2 are the two major and different ways of prolactin preventing apoptosis.

In conclusion, we have found that an increasing of ODC activity is another way of prolactin preventing MTX-induced apoptosis. Further, this induction of ODC activity further enhances the expression of Bcl-2 sufficiently strongly to bring about the anti-apoptotic function. As well, ODC activity and the manufacture of the polyamine pathway have already been important targets for therapeutic intervention in many types of cancer. This novel finding may be helpfully in providing therapeutic suggestions for combination of treatment in diseases.

Figure

Fig. 1. Prolactin prevents MTX-induced apoptosis in a dose-dependent manner.

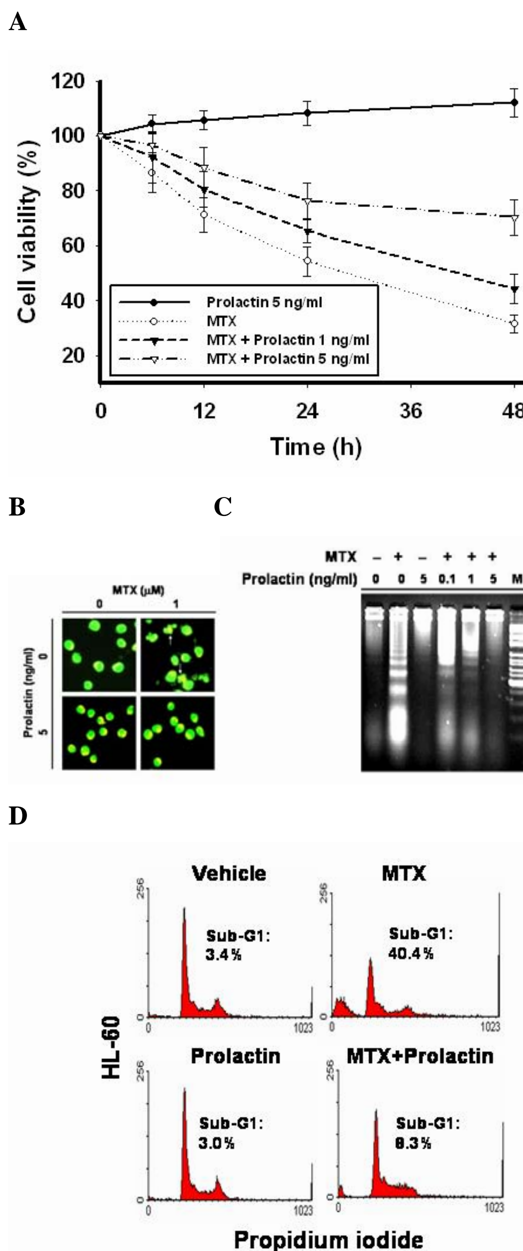
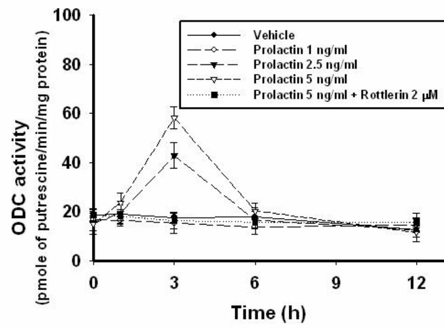


Fig. 2. Prolactin increases ODC activity via the PKCδ pathway in a dose-dependent manner, but does not affect mRNA expression.

A



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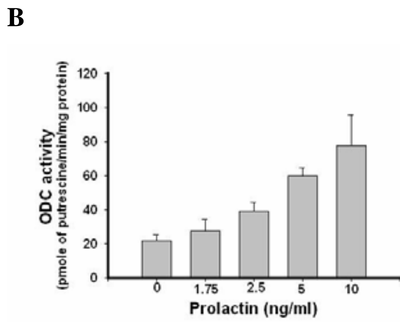

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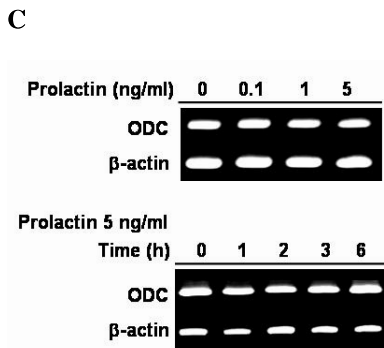
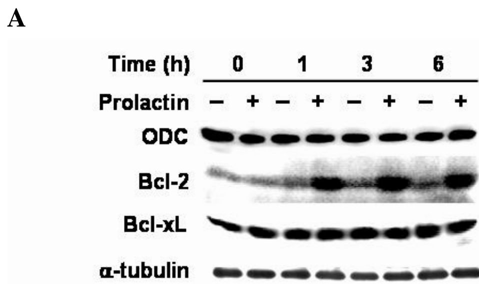


Fig. 3. Prolactin doesn't increase the protein levels of ODC and Bcl-xL, but does upregulate Bcl-2.

A



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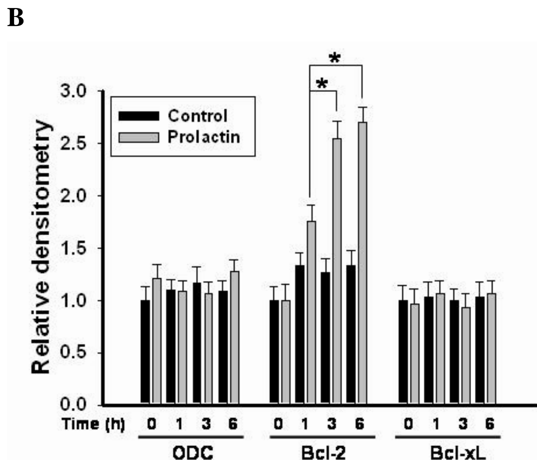
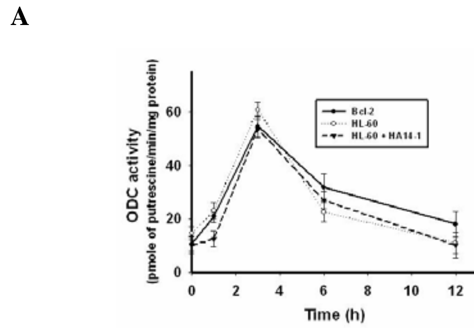


Fig. 4. Overexpression of Bcl-2 has no effect on the activity of ODC and its protein level.

A



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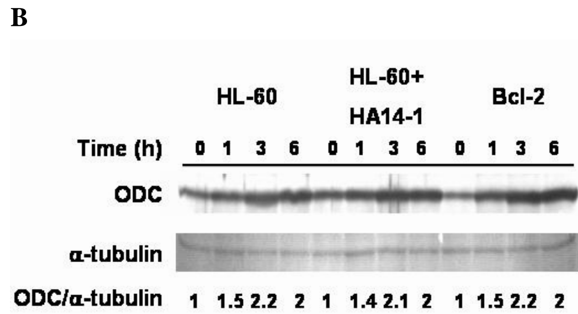


Fig. 5. Overexpression of ODC upregulates Bcl-2.

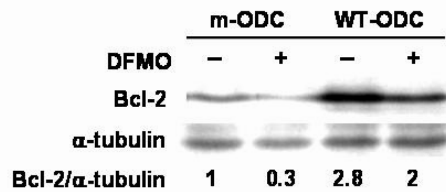
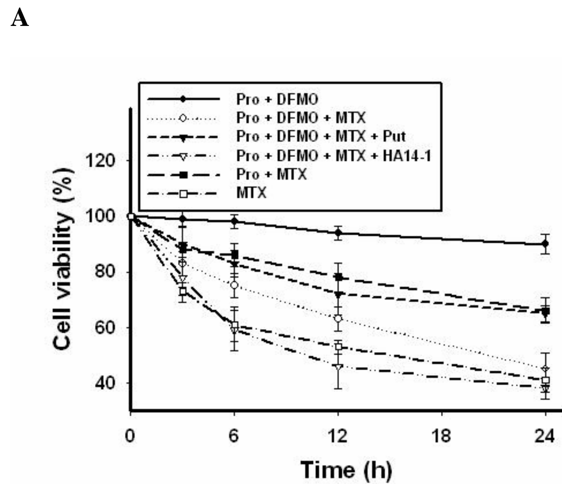


Fig. 6. The effects of DFMO and HA14-1 on the anti-apoptotic function of prolactin.

A



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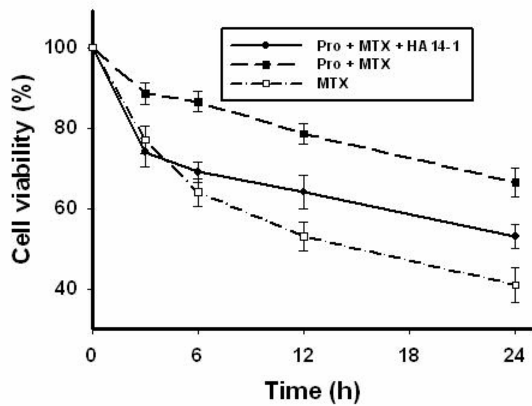
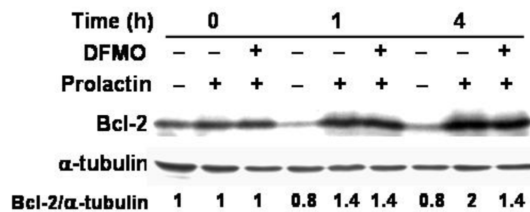


Fig. 7. The effect of DFMO on upregulation of Bcl-2 following prolactin stimulus.

A



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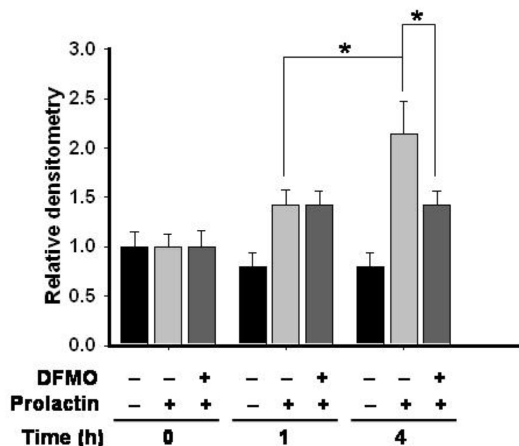

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成果報告 貳 （已發表於2006, *Apoptosis*）

Abstract

Ornithine decarboxylase (ODC) plays an essential role in various biological functions, including cell proliferation, differentiation and cell death. However, how it prevents the cell apoptotic mechanism is still unclear. Previous studies have demonstrated that decreasing the activity of ODC by difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, causes the accumulation of intracellular reactive oxygen species (ROS) and cell arrest, thus inducing cell death. These findings might indicate how ODC exerts anti-oxidative and anti-apoptotic effects. In our study, tumor necrosis factor alpha (TNF-α) induced apoptosis in HL-60 and Jurkat T cells. The kinetic studies revealed that the TNF-α-induced apoptotic process included intracellular ROS generation (as early as 1 h after treatment), the activation of caspase 8 (3 h), the cleavage of Bid (3 h) and the disruption of mitochondrial membrane potential ($\Delta\psi_m$) (6 h). Furthermore, ROS scavengers, such as glutathione (GSH) and catalase, maintained $\Delta\psi_m$ and prevented apoptosis upon treatment. Putrescine and overexpression of ODC had similar effects as ROS scavengers in decreasing intracellular ROS and preventing the disruption of $\Delta\psi_m$ and apoptosis. Inhibition of ODC by DFMO in HL-60 cells only could increase ROS generation, but did not disrupt $\Delta\psi_m$ or induce apoptosis. However, DFMO enhanced the accumulation of ROS, disruption of $\Delta\psi_m$ and apoptosis when cells were treated with TNF-α. ODC overexpression avoided the decline of Bcl-2, prevented cytochrome c release from mitochondria and inhibited the activation of caspase 8, 9 and 3. Overexpression of Bcl-2 maintained $\Delta\psi_m$ and prevented apoptosis, but could not reduce ROS until four hours after TNF-α treatment. According to these data, we suggest that TNF-α induces apoptosis mainly by a ROS-dependent, mitochondria-mediated pathway. Furthermore, ODC prevents TNF-α-induced apoptosis by decreasing intracellular ROS to avoid Bcl-2 decline, maintain $\Delta\psi_m$, prevent cytochrome c release and deactivate the caspase cascade pathway.

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Liu GY, Hung YC, Hsu PC, Liao YF, Chang WH, Tsay GJ, Hung HC (2005): Ornithine decarboxylase prevents tumor necrosis factor alpha-induced apoptosis by decreasing intracellular reactive oxygen species. Apoptosis 10:569-81.

成果報告 參 （已發表於2006, *Apoptosis*）

Methotrexate (MTX), a folate antagonist, was developed for the treatment of malignancies, and is currently used in rheumatoid arthritis (RA) and other chronic inflammatory disorders. It has been proven in short-term and long-term prospective studies that low doses of MTX (0.75 mg/Kg/week) are effective in controlling the inflammatory manifestations of RA. Low-concentrations of MTX achieve apoptosis and clonal deletion of activated peripheral T cells. One of the mechanisms of the anti-inflammatory and immunosuppressive effects may be the production of reactive oxygen species (ROS). However, the drug resistance of MTX in malignancies remains poorly understood. Ornithine decarboxylase (ODC) plays an important role in diverse biological functions, including cell development, differentiation, transformation, growth and apoptosis. In our previous studies, ODC overexpression was shown to prevent TNFα-induced apoptosis via reducing ROS. Here, we also investigated one mechanism of MTX-induced apoptosis and of drug resistance as to the anti-apoptotic effects of ODC during MTX treatment. We found MTX could induce caspase-dependent apoptosis and promote ROS generation together with disrupting the mitochondrial membrane potential ($\Delta\psi_m$) of HL-60 and Jurkat T cells. Putrescine and ROS scavengers could reduce MTX-induced apoptosis, which leads to the loss of $\Delta\psi_{m,}$ through reducing intracellular ROS. Overexpression of ODC in parental cells had the same effects as putrescine and the ROS scavengers. Moreover, ODC overexpression prevented the decline of Bcl-2 that maintains $\Delta\psi_m$, the cytochrome c release and activations of caspase 9 and 3 following MTX treatment. The results demonstrate that MTX-induced apoptosis is ROS-dependent and occurs along a mitochondria-mediated pathway. Overexpressed ODC cells are resistant to MTX-induced apoptosis by reducing intracellular ROS production.

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Huang CC, Hsu PC, Hung YC, Liao YF, Liu CC, Hour CT, Kao MC, Tsay GJ, Hung HC, **Liu GY** (2005): Ornithine decarboxylase prevents methotrexate-induced apoptosis by reducing intracellular reactive oxygen species production. Apoptosis 10:895-907. (Corresponding author)

成果報告 肆 （已發表於2006, *Apoptosis*）

Antizymes delicately regulate ornithine decarboxylase (ODC) enzyme activity and polyamine transportation. One member of the family, antizyme-1, plays vital roles in molecular and cellular functions, including developmental regulation, cell cycle, proliferation, cell death, differentiation and tumorigenesis. However, the question of how does it participates in the cell apoptotic mechanism is still unsolved. To elucidate the contribution of human antizyme-1 in haematopoietic cell death, we examine whether inducible overexpression of antizyme enhances apoptotic cell death. Antizyme reduced the viability in a dose- and time-dependent manner of human leukemia HL-60 cells, acute T leukemia Jurkat cells and mouse macrophage RAW 264.7 cells. The apoptosis-inducing activities were determined by nuclear condensation, DNA fragmentation, sub-G_1 appearance, loss of mitochondrial membrane potential ($\Delta\psi_m$), release of mitochondrial cytochrome *c* into cytoplasm and proteolytic activation of caspase 9 and 3. Following conditional antizyme overexpression, all protein levels of cyclin-dependent kinases (Cdks) and cyclins are not significantly reduced, except cyclin D, before their entrance into apoptotic cell death. However, introduced cyclin D1 into Jurkat T tetracycline (Tet)-On cell system still couldn't rescue cells from apoptosis. Antizyme doesn't influence the expression of tumor suppressor p53 and its downstream p21, but it interferes in the expressions of Bcl-2 family. Inducible antizyme largely enters mitochondria resulting in cytochrome *c* release from mitochondria to cytosol following Bcl-xL decrease and Bax increase. According to these data, we suggest that antizyme induces apoptosis mainly through mitochondria-mediated and cell cycle-independent pathway. Furthermore, antizyme induces apoptosis not

only by Bax accumulation reducing the function of the Bcl-2 family, destroying the $\Delta\psi_m$, and releasing cytochrome *c* to cytoplasm but also by the activation of apoptosomal caspase cascade.

References

Liu GY, Liao YF, Hsu PC, Chang WH, Hsieh MC, Lin CY, Hour TC, Kao MC, Tsay GJ, Hung HC (2006): Antizyme, a natural ornithine decarboxylase inhibitor, induces apoptosis of haematopoietic cells through mitochondrial membrane depolarization and caspases' cascade. Apoptosis 11:1773-88.

出席國際學術會議心得報告

計畫編號	95-2320-B-040-020-
計畫名稱	鳥胺酸去羧化酶對抗計畫性細胞死亡之機制
出國人員姓名 服務機關及職稱	劉光耀；中山醫學大學免疫學研究所；副教授兼所長
會議時間地點	2007/7/7~2007/7/12；奧地利 維也納
會議名稱	(中文) 2007 年歐洲生物化學學會聯盟會議(分子機制) (英文) FEBS 2007 Congress (Molecular Machines)
發表論文題目	Establishment of the dual luciferase frameshift assay in the acute T leukemia Jurkat cells

一、參加會議經過

筆者來到奧地利維也納，其氣候為涼爽，日照時間至晚上九點天黑。城市有許多的教堂與古蹟，非常漂亮，各式搭乘交通工具非常便利，是一個適合舉行國際會議的地方。

第三十二屆歐洲生物化學學會聯盟會議:分子機制於奧地利維也納(Vienna-Austria)舉行，自 2007 年 7 月 07 日起至 12 日止，為期六天，共有來自世界各地學者、學生與廠商逾數千人與會，會中壁報展示的時間 7 月 08 日起至 12 日止。議程包括專題演講與壁報展示，其中的專題演講每天分成五大主題，由相關領域頂尖學者提供研究上的第一手資訊和與會研究者分享。本次會議所涵蓋之層面探討從細胞核至細胞膜上之分子機制、利用系統生物瞭解蛋白質的分子機制，以及探究 chemogenomics 提升和加速藥物應用於臨床的可能性。尤其這個會議是歐洲歷史悠久與會員眾多的生化學會舉辦，參與之論文均為上上之選。筆者在這次的會議中發表了一篇研究論文，以壁報展示，內容是有關『Establishment of the dual luciferase frameshift assay in the acute T leukemia Jurkat cells』，藉由與會之機會，將研究成果與各國學者專家交換心得及經驗，提升研究品質。我們的參與不僅提供互相觀摩的機會，更可提升國內研究之發展及掌握世界生命科學發展的脈動。

二、與會心得

今年會議邀請兩位諾貝爾得主，第一位是 Blobel 先生作為開幕的主講者，其演講有關 Nucleoporins 如何將細胞核的大分子運出至細胞質之分子機制，說明蛋白質間的相互作用與結構的特性，微妙連結將分子一步步由細胞核送到細胞質。第二位是 Ciechanover 先生作為閉幕的主講者，其演說以 Ubiquitin 相關之降解蛋白質系統，說明與人類疾病的關連性，並且如何發展此方面的藥物，治療疾病。從這兩位諾貝爾得主的演講清楚知道他們對於研究的執著與努力，這些都是值得筆者效法的。

讓筆者聯想到自己所研究的鳥胺酸去羧化酶，其為第一個以 Ubiquitin 相關降解蛋白質機制之外進行降解的蛋白質，而調節其降解為抑酶，近年來有科學家也陸續其他蛋白質可能受

到抑酶調節進行降解，如果能夠理清抑酶之降解蛋白質系統，也說明與人類疾病的相關，發展此方面的藥物，治療疾病，這是未來所能進行的研究。

此外，Quatitative proteomicsc 和 Systems biology 的演講中，Kubinyi、Mestres 與 Scherrer 三位學者介紹 Chemogenmics 的研究與發展情況，以前確定藥物的可用性只能將其合成，進行分析，再慢慢經過試驗，最後只有少部分才能真正使用於臨床，而現在的研究者利用蛋白質學與系統生物了解蛋白質的結構與 Ligand-Base 推斷可能的效率，之後再進行實驗，以擴大成效性。

三、建議

與國際性學術會議對於研究者能夠增廣見聞，同時吸收新知，更能和研究同一領域或不同方向之外國學者互相討論切磋，提升研究的廣度，以能應用治療疾病。希望國科會對於參加此類國際知名大型會議的學者，更加大力支持，以增進研究的國際競爭力。

四、攜回資料名稱及內容

此次會議攜回會議論文集一本、課程手冊一本及相關期刊數種單行本，內容包括全部演講與壁報論文之題目和摘要，分別包括: Nuclear world、Transport machineries、Signal transdution、Metabolism & Energetics 和 Quantitative biology 等。

五、其他

這次研討會筆者多停留兩天於奧地利，研討會結束後，筆者起程至莫札特的故鄉薩爾斯堡，到處充滿莫札特的訊息。從維也納到薩爾斯堡，筆者感覺奧地利人對於他們周圍古蹟都非常愛護，更讓筆者思考對於周邊環境的關心程度。

六、摘要及壁報

Establishment of the dual luciferase frameshift assay in the acute T leukemia Jurkat cells

Guang-Yaw Liu[1], Chien-Yu Lin[1] and Hui-Chih Hung[2]

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[2] Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan, ROC

Abstract

Many viral, microbiologic and botanic mRNAs can make translating ribosome change resulted in reading frame switch. However, antizyme frameshifting uniquely exists in the mammalian cells. Ribosomal frameshift is a translational control mechanism where specific signals in the mRNA instruct the ribosome to change reading frame at certain efficiency, to continue translation in the new frame and to produce novel protein. Antizyme plays the vital roles in molecular and cellular functions, including developmental regulation, cell cycle, proliferation, cell death, differentiation and tumorigenesis. In our previous study, the overexpression of antizyme induces cell apoptosis through the mitochondrial-mediated and caspase-dependent pathway (Liu et al., 2006 Apoptosis).

Herein, a new and stable reporter system is developed for measuring the translation coupling efficiency of frameshifting. We establish the plasmid construct employed the dual luciferase proteins, renilla and firefly that can be assayed independently and easily for studying the frameshifting mechanism in the acute Jurkat T cells. Subsequently, the system of stable mammalian frameshift assay can be applied to select the specific fragments of sequences, the upstream regulators, and both in vivo and in vitro drug screenings of the +1 translational frameshift of antizyme or new genes.

Establishment of the dual luciferase frameshift assay in the acute T leukemia Jurkat

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2. National Chung-Hsing University, Taichung, Taiwan

ABSTRACT

Many viral, microbiologic and botanic mRNAs can make translating ribosome change resulted in reading frame switch. However, antizyme frameshifting uniquely exists in the mammalian cells. Ribosomal frameshift is a translational control mechanism where specific signals in the mRNA instruct the ribosome to change reading frame at certain efficiency, to continue translation in the new frame and to produce novel protein. Antizyme plays the vital roles in molecular and cellular functions, including developmental regulation, cell cycle, proliferation, cell death, differentiation and tumorigenesis. In our previous study, the overexpression of antizyme induces cell apoptosis through the mitochondrial-mediated and caspase-dependent pathway (Liu et al., 2006 Apoptosis). Herein, a new and stable reporter system is developed for measuring the translation coupling efficiency of frameshifting. We establish the plasmid construct employed the dual luciferase proteins, renilla and firefly that can be assayed independently and easily for studying the frameshifting mechanism in the acute Jurkat T cells. Subsequently, the system of stable mammalian frameshift assay can be applied to select the specific fragments of sequences, the upstream regulators, and both *in vivo* and *in vitro* drug screenings of the +1 translational frameshift of antizyme or new genes.

RESULTS

Figure 1. Site-directed mutagenesis of Luc2P in firefly luciferase start codon

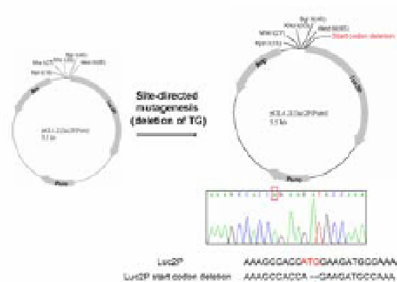


Figure 2. pCMV or pTRE promoter construct introduces into the dual luciferase frameshift assay

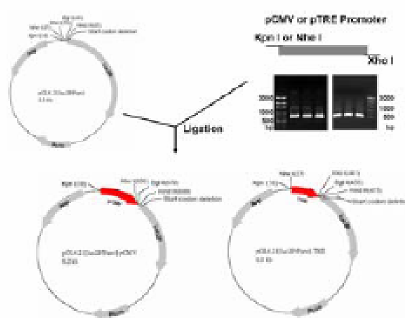


Figure 3. *hRluc* luciferase gene construct introduces into the dual luciferase frameshift assay

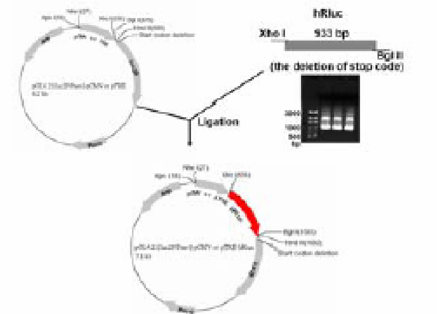


Figure 4. *Antizyme (AZ)* and *mAZ* gene constructs introduce into the dual luciferase frameshift assay

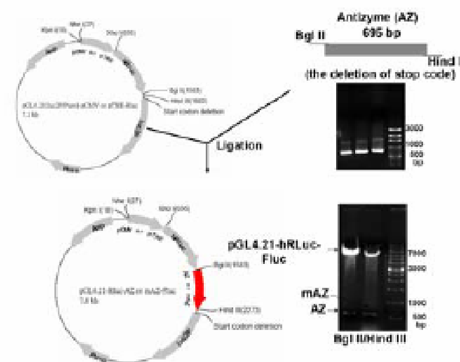
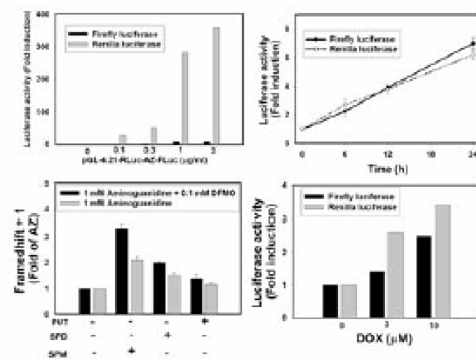


Figure 5. Assay the dual luciferase activity in transient or stable transfection system



DISCUSSION

We have established the plasmid construct employed two luciferase proteins, renilla (hRluc) and firefly (Fluc2P), that can be assayed independently to detect ribosomal frameshift. Subsequently, the system of stable mammalian frameshift assay can be applied to identify the fragments of specific sequences or the upstream regulators which participate the processes of ribosomal frameshift, and screen drugs or proteins that interfere with ribosomal frameshift both *in vivo* and *in vitro*.

七、大會議程

Programme Overview - 32nd FEBS Congress Vienna — Molecular Machines

SATURDAY - *July 7 - Evening*

Opening Ceremony
Award FEBS Diplomme d'Honeurs
17:00 - 19:00 - Main Auditorium

Keynote Opening Lecture
Günter Blobel - US
Nobel Laureate in Physiology &
Laudatio: Günther Kreil
19:00 - 20:00 - Main Auditorium

Welcome Mixer & Cocktail
20:00 - 22:00 - Main Auditorium & Exhibition

	SUNDAY - *July 8*	**MONDAY** - *July 9*	**TUESDAY** - *July 10*	**WEDNESDAY** - *July 11*	**Thursday** - *July 12*
Nuclear World	*A1* **Chromosomes**	*A2* **Chromatin**	*A3* **Nuclear Dynamics**	*A4* **RNAis & microRNAs**	*A5* **Transcription & RNA Processing**
8:30 - 11:30 - Symposium A	*Chair: Jan-Michael Peter - AT*	*Chair: N.N.*	*Chair: Roland Foisner - AT*	*Chair: Marjori Matzke - AT*	*Chair: Andrea Barta - AT*
	John F. Marko - US	John Diffley - UK	Andrew Belmont - US	Witek Filipowicz - CH	Benoit Coulombe - CA
	Susanne Lens - NL	Patrick Cramer - DE	Amanda Fisher - UK	Javier Martinez - AT	Alberto R. Kornblihtt - AR
	Jan Karlseder - US	Yoshinori Watanabe - JP	Jiri Bartek - DK	Reuven Agami - NL "EMBO YIP Lecture"	Karla Neugebauer - DE
	Conly Rieder - US	Danesh Moazed - US	Joan Steitz - US	Cecilia Arraiano - PT	Reinhard Lührmann - DE
	N.N.	Marjori Matzke - AT	Ulrike Kutay - CH	Olivier Voinnet - FR "EMBO YIP Lecture"	Elisa Izaurralde - DE "FEBS Journal Lecture"
Transport Machineries	*B1* **Intracellular Trafficking**	*B2* **Secretion & Sorting**	*B3* **Organelle Biogenesis**	*B4* **Membrane Transporters**	*B5* **Cell-To-Cell Transport & Motility**
8:30 - 11:30 - Symposium B	*Chair: Fritz Kragler - AT*	*Chair: Felix Wieland - DE*	*Chair: Cecile Brocard - AT*	*Chair: Peggy Stoll-Bergner - AT*	*Chair: Elisabeth Waigmann - AT*
	Markus Aebi - CH	Guy Cornelis - CH	Iain Mattaj - DE	Yuichi Sugiyama - JP	Tone Tonjum - NO
	Jürgen Knoblich - AT	Jorge Galan - US	Jürgen Soll - DE	Kaspar Locher - CH	Colin Hughes - UK
	Jennifer Lippincott-Schwartz - US	Jeffrey E. Gerst - IL	Nikolaus Pfanner - DE	Vinzenz Unger - US	William Lucas - US
	Alberto Luini - IT	Marino Zerial - DE	Ralf Erdmann - DE	Tom Walz - US	Suzanne Eaton - DE
	Graham Warren - AT "Max F. Perutz Lecture"	Harald Stenmark - NO	Daniel Klionsky - US	Raimund Dutzler - CH "EMBO YIP Lecture"	Hendrik Korswagen - NL
Signal Transduction	*C1* **Signaling & Development**	*C2* **Cancer & Apoptosis**	*C3* **Host-Pathogen Interaction**	*C4* **Stress Response**	*C5* **Regulated Turnover**
8:30 - 11:30 - Symposium C	*Chair: Manuela Baccarini - AT*	*Chair: Wolfgang Mikulits - AT*	*Chair: Mathias Müller - AT*	*Chair: Pavel Kovarik - AT*	*Chair: Andrea Pichler - AT*
	Gerd Jürgens - DE	Michael Hengartner - CH	Walther Mothes - US	Regine Hengge-Aronis - DE	Elena Conti - DE
	Barry Dickson - AT	Boris Turk - SL	Carmen Buchrieser - FR	Francesc Posas - ES	Brenda Schulman - US
	David Feldheim - US	George Mosialos - GR	Thomas Decker - AT	Heribert Hirt - AT	Dieter Wolf - DE
	Rudi Grosschedl - DE	Mariano Barbacid - ES	Stefan Kaufmann - DE	Axel Behrens - UK "EMBO YIP Lecture"	Daniel Finley - US
	Monique Gangloff - UK	Gerhard Christofori - CH	Arturo Casadevall - US	Fabrizio Chiti - IT "EMBO YIP Lecture"	Frauke Melchior - DE
Metabolism & Energetics	*D1* **Microbial Metabolism**	*D2* **Electron Transport & Multienzyme Complexes**	*D3* **Drug Targets**	*D4* **Molecular Clocks**	*D5* **Metabolic Networks**
8:30 - 11:30 - Symposium D	*Chair: Günther Koraimann - AT*	*Chair: Andreas Hartig - AT*	*Chair: Peter Chiba - AT*	*Chair: Arndt von Haeseler - AT*	*Chair: Stefan Thurner -*
	Dieter Oesterhelt - DE	Roland Lill - DE	Greg Petsko - US	Michael Brunner - DE	Uwe Sauer - CH
	Michael Wagner - AT	Carola Hunte - DE	Anton Stütz - AT	David Lloyd - UK	Michael Hall - CH
	Kalervo Hiltunen - FI	Jean-David Rochaix - CH	Axel Ullrich - DE	Michael Rosbash - US	Lothar Willmitzer - DE
	Franz-Ulrich Hartl - DE	Peter Csermely - HU	Hugo Kubinyi - DE	Uli Schibler - CH	Joan Guinovart - ES
	Barbara Reinhold-Hurek - DE	Richard Perham - UK	N.N.	Seth Jon Davis - DE	Csaba Pal - HU
Quantitative Biology	*E1* **New age "-OMICS"**	*E2* **Systems Biology** - *Astra Zeneca Symposium*	*E3* **Dynamics of Complexes**	*E4* **Predictive Modeling**	*E5* **Molecular Recognition**
8:30 - 11:30 - Symposium E	*Chair: David Kreil - AT*	*Chair: Karl Kuchler - AT*	*Chair: Thomas Marlovits - AT*	*Chair: Leonie Ringrose - AT*	*Chair: Robert Konrat - AT*
	John Mattick - AU	Judith Armitage - UK	Wolfgang Baumeister - DE	Stefan Schuster - DE	Norbert Polacek - AT
	Charlie Boone - CA	Hans Westerhoff - NL	Arnold Driessen - NL	Edda Klipp - DE	Matthias Wilmanns - DE
	Katsuhiko Shirahige - JP	Ursula Klingmüller - DE	Holger Stark - DE	Bela Novak - UK	Andrea Musacchio - IT
	Marius Ueffing - DE	Jim Ferrell - US	Karl-Peter Hopfner - DE	Marc Vidal - US	Alexandre Bonvin - NL
	Jan Ellenberg - DE	Jaroslav Stark - UK	Tom Steitz - US	Jacky Snoep - ZA	Frédéric Allain - CH
Plenary Medal Lectures	**KREBS Medal Lecture**	**BÜCHER Medal Lecture**	**Plenary AWARD SESSION**	**DATTA Medal Lecture**	**Keynote Closing Plenary Lecture**
11:40 - 12:40 - Main Auditorium	Tom Rapoport - US *Chair & Laudatio: Jan-Michael Peters & Iain Mowbray*	Kim Nasmyth- UK *Chair & Laudatio: Karl Kuchler & Felix Wieland*	FEBS Lett / FEBS J / YSF2007 *Chair: Felix Goñi & Julio Celis*	Venki Ramakrishnan- UK *Chair & Laudatio: Andrea Barta & Israel Pecht*	Aaron Ciechanover - IL *Nobel Laureate in Chemistry 2004* *Laudatio: Frauke Melchior*

Parallel Lunchtime Tutorials & Events & Corporate Seminars					
12:45 - 14:00 - **Main Auditorium**	KinderUni Wien Lecture in German **Angelika Amon - US** - *Was ist Krebs?* Science for Kids - Childrens University Vienna	**KinderUni Wien - Speaker Interviews** Kid Journalists interview FEBS2007 Speakers	**KinderUni Wien - Interviews** Kid Journalists interview FEBS2007 Spe	KinderUni Wien Lecture in German Arndt von Haeseler - AT *Wieso sind Gene verschieden?* Science for Kids - Childrens University Vienna	**13:00 - 14:00 - Main Auditorium** **Closing Ceremony**
12:45 - 14:00 - **Room A**	**Company Lunch Seminars**	**Biacore Lunch Seminar**	12:45 - 14:00 - FEBS Room **Meeting FEBS Congress Organizers**	**Perkin Elmer Lunch Seminar**	
Parallel Afternoon Sessions	**Science Workshops & Special Programmes & Workshops & Policy-Making**				
14:00 - 17:00 - **Room B**	**Data Mining/Bioinformatics Tutorial** 14:00 - 17:00	**Data Mining/Bioinformatics Tutorial** 14:00 - 17:00		**Data Mining/Bioinformatics Tutorial** 14:00 - 17:00	
14:00 - 15:30 **Lecture Hall A**	**Lipids: From Diseases to Aging** *Chair: Georg Wick - AT* Elisabeth Steinhagen-Thiessen - DE Rudolf Zechner - AT Joseph Witztum - US	**Chemogenomics & Drug Discovery** *Chair: Gerhard Ecker - AT* Adriano Henney - UK Jordi Mestres - ES N.N.		**Bioinformatics - Evolution & Databases** *Chair: Arndt von Haeseler - AT* Doron Lancet - IL William Martin - DE David Kreil - AT Philipp Khaitovich - CN	
14:00 - 15:30 **Lecture Hall B**	**Cytoskeleton & Motility** *Chair: Vic Small - AT* Michael Way - UK Stefan Westermann - AT Darren Gilmour - DE	**Quantitative Proteomics & Networks** *Chair: Giulio Superti-Furga - AT* Christopher Overall - CA Blagoy Blagoev - DK John J.M. Bergeron - CA		**Integrative Structural Biology** *Chair: Werner Kühlbrandt - DE* Christian Spahn - DE Enrico Schleiff - DE Rob Russel - DE	
14:00 - 15:30 **Lecture Hall C**	**FEBS/EMBO - Science & Society Workshop** **Aging of the Brain** *Chair: Frederico Mayor* Beyreuther Konrad - DE *"EMBO Speaker"*	**FEBS-EMBO Workshop on WISE** **Why Supporting Careers of Women?** *Chair: Saskia van der Vies - NL* Gerlind Wallon - DE Gertraud Oberzaucher - AT Maaike Romijn - NL		**Education & Teaching Workshop** **Does Education Meet Future Challenges?** *Chair: Ed Wood - UK* David Bunton - UK Alexander von Gabain - AT Martino Picardo - UK	
14:00 - 15:30 **Lecture Hall D**	**ELSI/ERC/FP7 Policy Workshop** *Chair: Luc van Dyck - DE* Julio Celis - DK Robert-Jan Smits -BE	**EC Cancer Research - Visions & Dreams** *Chair: Julio Celis - DK* Mariano Barbacid - ES Erwin Wagner - AT Manuel Hallen - BE Frank Gannon - IR	FREE AFTERNOON	**Grant Writing & Career Workshop** *Chair: Daniela Corda -IT* Dius Lennon - BE Karl Kuchler - AT	FEBS COUNCIL MEETING
14:00 - 15:30	**Reserve Slot**	**Communicating Science & Media Contest** *Chair: Karl Kuchler - AT* Elisabeth Weigmann - AT Andrew Moore - DE		**Reserve Slot**	
Parallel Sessions	**Afternoon Plenary Talks & Poster Sessions**				
15:40 - 16:30 **Main Auditorium**	**EMBO Plenary Lecture** **Titia de Lange** - US *Chair & Laudatio: Jan Karbeder - US*	**IUBMB Plenary Lecture** **Angelika Amon** - US *Chair & Laudatio: Egon Ogris & Jacques-Henry Weil (IUBMB)*		**PABMB Plenary Lecture** **Mike Tyers** - CA *Chair & Laudatio: Charlie Boone & Juan Cazzulo (PABMB)*	
15:30 - 18:30 **POSTER Hall**	**Free POSTER PreViewing** 15:30 - 18:30	**POSTER Viewing - Poster Numbers Presence** 16:30 - 17:30 ODD & 17:30-18:30 - EVEN **Blackwell/Elsevier Wine Tasting at the FEBS Booth** 16:30 - 18:30 - *"Meet the FEBS Editors"*		**POSTER Viewing - Poster Numbers Presence** 16:30 - 17:30 EVEN - 17:30-18:30 - ODD	
Parallel Evening Events	**Events Downtown Vienna - Outside of the Venue & Social Activities**				
19:00 - 23:30	**Students Meet Speakers in Bars 1**	**Students Meet Speakers in Bars 2**		**Students Meet Speakers in Bars 3**	
19:00 - 23:30	**Special Concert in the Musikverein** **Hugo-Wolf Quartett**	**Science in Public Places** ***Re-emerging Diseases - Global Threats?***	**Gala Dinner Reception Rathaus** Viennese Dancing	**Science in Public Places** ***Stem Cells & Society***	
	Wiener Musikverein *Sponsored by UNIQA*	*Birgit Dahlheimer* Alexander von Gabain - AT Stephan Kaufmann - CH Doug Holtzman - US ***Natural History Museum***	***Vienna City Hall***	*Klaus Taschwer* Mary Maxon - US Meinrad Busslinger - AT ***Museum of Modern Art***	**FEBS COUNCIL DINNER** 20:00 - 23:00 Council Representatives Only
20:30 - 23:30		**FEBS Dinner - Medal Lecturers &Diplomme d'Honneurs** By Personal Invitation Only			