



如何撰寫研究 計畫

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研究計畫補助要點

學門計畫通過率約在40%，多年期計畫至少應在排序前20%，獲第2件計畫補助須至少排序在前12%，獲博士後研究員額須至少排序前15%。因此，在評審表格，設定極力推薦(88分以上；前12%)、優先推薦(87-85分；前20%)、推薦(84-80分；20-40%)、不推薦(79分以下)。

研究計畫審查步驟

- 每個計劃由每位複審委員推薦2位初審委員
- 每個計劃分別由2位初審委員及2位複審委員獨立審查(複審可看到初審的評語，但無法看到成績)
- 兩位初審成績相差8~14分，則再增第三位初審委員提供成績
- 兩複審成績相差8~15分，初複審平均差8~14分，則再複審會議提出討論
- 每位複審委員的成績會再做一次校正

計畫審查計分

- 過去五年研究成果 (30%)
新進人員 (20%)
- 計畫審查要點 (70%)

研究計畫補助審查要點

- 計畫的創新性及重要性
- 計畫的可行性
- 主持人的研究表現
- 計畫的撰寫

計畫的先前佈署

- 加速論文發表
- 盡早準備 Preliminary data

研究計畫內容：

研究計畫之背景及目的。請詳述本研究計畫之背景、目的、**重要性**及國內外有關本計畫之研究情況、重要參考文獻之評述等。本計畫如為整合型研究計畫之子計畫，請就以上各點分別述明與其他子計畫之相關性。

研究方法、進行步驟及執行進度。請分年列述：1.**本計畫採用之研究方法與原因**。2.預計可能遭遇之困難及解決途徑。3.重要儀器之配合使用情形。4.如為整合型研究計畫，請就以上各點分別說明與其他子計畫之相關性。5.如為須赴國外或大陸地區研究，請詳述其必要性以及預期成果等。

預期完成之工作項目及成果。請分年列述：1.預期完成之工作項目。2.**對於學術研究、國家發展及其他應用方面預期之貢獻**。3.對於參與之工作人員，預期可獲之訓練。4.本計畫如為整合型研究計畫之子計畫，請就以上各點分別說明與其他子計畫之相關性。

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研究計畫內容（以中文或英文撰寫）

This proposed research project is the extension of our previous grant proposal (2015-2018). The findings from our previous studies of “interaction between female sex hormones and folic acid (FA)” by MOST 104-2320-B-038-030-MY3 have been published in three papers in Scientific Reports (Kuo et al., 2015), Cancer Letters [Kuo and Lee, 2016] and Acta Physiologica [Lee et al., 2017], respectively, and one manuscript is under review.

FA has been frequently used as a nutritional supplement for folate. Folate deficiency has been related to various human diseases, including atherosclerosis, anemia, neural tube defects, neuropsychiatric disorders, and cancer. We previously uncovered a novel action of FA with an anti-angiogenic activity [Lin et al., 2012; Hou et al., 2013]. Inhibitions of angiogenesis as well as cancer cell proliferation and migration are important strategies for cancer therapy. We also showed that FA can reduce proliferation of cultured COLO-205 and cause regression of COLO-205 tumor bearing SCID mice [Kuo et al., 2015]. Surprisingly, we found that Org 31710, a progesterone receptor (PR) specific antagonist, can prevent the FA-induced anti-proliferation in colorectal cancer cell lines [Kuo and Lee, 2016]. These findings suggest that the expression of PR might affect the actions of FA on cancer cell behaviors. Our recent studies showed that female sex hormone receptors formed a complex with FA receptor (FR) and cSrc, and female sex hormones can interfere with the FA-induced anti-angiogenesis [Lee et al., 2017]. Accordingly, the aims of this new grant proposal will be focused on the study of how PR might affect the FA's action on cancer growth and metastasis. Three specific aims of the new grant proposal are shown as below:

Specific Aim 1: *In vitro* studies of the involvement of PR in the FA-induced proliferation and migration inhibition in cancer cell lines

Specific Aim 2: Molecular mechanisms underlying PR-regulated FA's effects on cancer cell proliferation and migration

Specific Aim 3: *In vivo* studies of the involvement of PR in the FA-affected tumor growth and metastasis

Background and significance

- **Folic acid (FA)**
- **Effects of FA on the development of cancer**
- **Folic acid receptors (FRs)**
- **Progesterone receptor (PR)**
- **Role of cSrc expression and activation in human cancer**
- **Regulation of cSrc activity**
- **Interaction among FR, PR, and cSrc**

Materials and Methods

Experimental design

- **Specific aims**
 - **Rationale**
 - **Experimental design**
(Three specific issues will be addressed in the study)
- **Preliminary results**
- **General procedures**

Specific Aim 1: *In vitro* studies of the involvement of PR in the FA-induced proliferation and migration inhibition in cancer cell lines

Rationale: Our previous studies have shown that blockade of the PR activity using Org 31710, a PR specific antagonist, abolished the FA-induced proliferation inhibition in COLO-205 cell line [Kuo and Lee, 2016]. Blockade of PR activity or knockdown of PR expression using PR antisense oligonucleotide also abolished the FA-induced activation of the signaling pathway involved in proliferation inhibition in colorectal cancer cell lines. These findings suggest that PR is required for FA-induced anti-proliferation in colorectal cancer cell lines. However, this is a general phenomenon or specific for colorectal cancers is still unclear. Accordingly, the aim of this study is to address this issue.

Experimental design: Three specific issues will be addressed in the study.

(1) Expression of FR and PR in cancer cell lines

Initially, we will perform RT-PCR to examine the expression of FR and PR in various cancer cell lines (colorectal, lung, liver, prostate, breast, and brain cancer cell lines). Our preliminary data showed that the PR mRNA was detected in COLO-205, HT-29, LoVo, PC3, DU145, LNCaP, T47D, Ca922, and PC9, but not DLD-1, HCT-116, Hep-G2 and Hep-3B (Figure 1). The expression of PR will be confirmed by Western blot analyses. Since the antibodies for detecting the isoforms of FR are still not available, the expression of FR isoforms in cancer cell lines will be identified using RT-PCR technique.

(2) Correlation between PR expression and cancer cell proliferation and migration

We will also examine the effects of FA on cancer cell proliferation and migration using MTT assay and Transwell or wound healing assay, respectively. The results will be correlated with the expression of PR in cancer cell lines. As shown in Figure 2, our preliminary data demonstrated that FA at a concentration of 10 μ M can reduce the proliferation of PR-positive colorectal cancer cell lines (COLO-205, HT-29 and LoVo), prostate cancer cell lines (DU-145, LNCaP and PC3), breast cancer cell line (T47D), and gingival squamous cell carcinoma (Ca922), but not PR-negative colorectal cancer cell lines (DLD-1 and HCT-116) and hepatoma cell lines (Hep-G2 and Hep-3B). Our preliminary data also showed that FA (10 μ M) reduced the migration activity of COLO-205, HT-29 and LoVo, which are PR-positive colorectal cancer cell lines, but

not DLD-1, a PR negative colorectal cancer cell line (Figure 3). These findings strongly suggest that FA-inhibited proliferation and migration of cancer cell lines only occurred in the PR-positive, but not PR-negative, cancer cell lines. We will further examine the effect of FA on cell proliferation and migration in more PR positive and negative cancer cell lines. In particular, we have only examined the effect of FA on the cell migration in one PR negative cell line so far.

(3) Effects of PR activity on the FA-induced proliferation and migration inhibition in cancer cell lines

Our previous research findings suggest that PR activation is required for FA-induced anti-proliferation in colorectal cancer cell lines. To verify whether this is a general phenomenon or specific for colorectal cancers, we will conduct similar experiments in some other PR positive and negative cancer cell lines. In this study, Org 31710, a PR specific antagonist, will be used to block the PR receptor activity, whereas PR antisense oligonucleotide will be applied to knock-down PR expression. The effect of blockade of PR action or expression on the FA-induced proliferation and migration inhibition in cancer cell lines will be examined using MTT assay and Transwell assay or wound healing assay, respectively. The role of PR activation on the FA-induced proliferation and migration inhibition in cancer cell lines will be confirmed by over-expression of exogenous PR in PR negative cancer cell lines. As illustrated in Figure 4, our preliminary data demonstrated that the anti-proliferation effect induced by FA in COLO-205, HT-29, PC-9, PC-3, DU-145, T47D and Ca922 can be abolished by pre-treatment with Org 31710, suggesting that PR activation is required for FA-induced proliferation inhibition in these cancer cell lines. We will confirm our assumption that PR activation is required for the FA-induced proliferation and migration in cancer cell lines. To achieve this goal, PR will be knocked-down in some PR positive cancer cell lines and over-expressed in some PR negative cancer cell lines, and then FA's effects on the proliferation and migration of these cell lines will be examined. We would expect that FA will not cause any significant effects on the proliferation and migration of these PR knocked-down cancer cell lines. On the other hand, FA will inhibit the proliferation and migration of PR over-expressed cancer cell lines.

Specific aim 1: In vitro studies of the involvement of PR in the FA-induced proliferation and migration inhibition in cancer cell lines

Rationale: Our previous studies have shown that blockade of the PR activity using Org 31710, a PR specific antagonist, abolished the FA-induced proliferation inhibition in COLO-205 cell line [Kuo and Lee, 2016]...These findings suggest that PR activation is required for the FA-induced anti-proliferation in colorectal cancer cell lines. However, this is a general phenomenon or specific for colorectal cancers is still unclear. Accordingly, the aim of this study is to address this issue.

Experimental design: Three specific issues will be addressed in the study

- (1) Expression of FR and PR in cancer cell lines**
- (2) Correlation between PR expression and cancer cell proliferation and migration**
- (3) Effects of PR activity on the FA-induced proliferation and migration inhibition in cancer cell lines**

Expression of FR and PR in cancer cell lines

Experimental design

Initially, we will perform **RT-PCR** to examine the expression of FR and PR in various cancer cell lines (colorectal, lung, liver, prostate, breast, and brain cancer cell lines). Our preliminary data showed that the PR mRNA was detected in COLO-205, HT-29, LoVo, PC3, DU145, LNCaP, T47D, Ca922, and PC9, but not DLD-1, HCT-116, Hep-G2 and Hep-3B (Figure 1). The expression of PR will be **confirmed by Western blot analyses**. Since the antibodies for detecting the isoforms of FR are still not available, the expression of FR isoforms in cancer cell lines will be identified using RT-PCR technique.

General Procedures

類似文章的Materials
and methods section

Cancer cell lines: In this study, colorectal cancer cell lines (COLO-205, HT-29, DLD-1 and HCT-116), prostate cancer cell lines (PC3 and DU145), lung cancer cell lines (A549 and HT1299), brain cancer cell lines (U87 and U373), liver cancer cell lines (Hep-G2 and Hep-3B), and breast cancer cell lines (T47D, MCF-7, and MB231) will be used.

Protein preparation and Western blot analysis: The protein extraction and Western blot analyses will be performed as described previously [Lee et al., 1998; Lin et al., 2001]. Briefly, the cells will be washed with PBS (pH 7.4), incubated with extraction buffer (Tris 10 mM, pH 7.0, NaCl 140 mM, PMSF 2 mM, DTT 5 mM, NP-40 0.5%, pepstatin A 0.05 mM and leupeptin 0.2 mM) with gentle shaking, and then centrifuged at 12,500 X g for 30 min. The cell extract will then be boiled in a ratio of 1:1 with sample buffer (Tris 100 mM, pH 6.8, glycerol 20%, SDS 4% and bromophenol blue 0.2%). Electrophoresis will be performed using 10-12% SDS-polyacrylamide gel (2 h, 110 V, 40 mA, 50 µg protein per lane). Separated proteins will be transferred to PVDF membranes (3 h, 40 V), treated with 5% fat-free milk powder to block the nonspecific IgGs, and incubated for 1 h with specific primary antibody. The blot will be then incubated with anti-mouse or -rabbit IgG linked to alkaline phosphatase (1:1000) for 1 h. Subsequently, the membrane will be developed with NBT/BCIP as a substrate. The quantitative data will be obtained by using a computing densitometer with Image-Pro plus software.

Plasmid and transient transfection: The transfection of siRNA or cDNA into cancer cell lines will be performed as previously described with minor modification [Lin et al., 2012]. Briefly, cancer cell lines will be seeded onto a six-well plate and transfected with siRNA or cDNA of candidate signaling molecules. After 48 h of transfection, the cell will be used for experiments indicated, and then processed for MTT assay or protein extraction and Western blot analysis. hPR1/pSG5 (form B) and hPR2/pSG5 (form A) are from Prof. Hinrich Gronemeyer (Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cedex, France).

Generation of stable PR-knocked-down HT-29 and PR-overexpression DLD-1: At least three clones of the HT-29 cell line stably expressing the siRNA or the DLD-1 cell line stably expressing the hPR1/pSG5 and hPR2/pSG5 were generated. All experiments were performed using multiple subclones of each cell line with consistent results. The PR-Si or PR-NT plasmids were transfected into HT-29 and the hPR1/pSG5 and hPR2/pSG5, or PR-NT were transfected into DLD-1, and stable integrants were selected at 72 h later with Geneticin (G418, 4 mg/mL). After 30 days in selective medium, three Geneticin (G418)-resistant clones, referred to as PR-Si or PR-Exp, were isolated.

Anticipated difficulty and alternative approaches

- 例如:你的實驗室沒有某個實驗技術
你可以找共同或協同主持人幫忙
- 例如:你預期某藥物的作用是透過 cSrc-mediated signaling pathway，結果 cSrc 的活性並未因藥物處理而有變化，你要換測試哪一條 signaling pathway

Preliminary results

最好對每一個specific aim
提出一兩個preliminary
results來支持其可行性

Figure Legends

Effects of FA on cancer cell proliferation. Treatment with FA (10 μ M) for 4 days inhibited the proliferation of PR positive, but not PR negative, cancer cell lines. The cell number was examined using MTT assay. Values represent the means $\pm$ s.e.mean. (n = 4). * $p < 0.05$ different from control group. Con, control; FA, folic acid; PR, progesterone receptor.

References

注意要有一些比較

updated References

Novelty

- being striking, original or unusual
- 避免 me too study (如法炮製)

Novelty

This proposed research project is the extension of our previous grant proposal. The findings from our previous studies of the effect of folic acid (FA) on angiogenesis sponsored by NSC 97-2320-B-038-033-MY3 have been published in *Angiogenesis* (Lin et al., 2012) and in *Biochemical Pharmacology* (Hou et al., 2012), respectively. **Demonstration of the anti-angiogenic activity of FA is a novel finding for the FA's action...** These findings suggest that female sex hormones might interfere with the actions of FA. Accordingly, the aims of this new grant proposal will be focused on studies of the interaction between female sex hormones and FA on their biological actions and treatment for diseases.

Significance

- 主持人應點出研究成果對於學術或臨床上的重要性
- 臨床議題的計畫較容易點出其重要性

計畫的可行性

- 主持人具有相關議題的文章發表
- 具備足夠的Preliminary data
- 嚴謹的實驗設計
- 擁有計畫執行的技術與設備
- 擁有適當的共同或協同主持人

Preliminary data

- 支持計畫執行的可行性
- 不能是已發表的Data

也不要放太多 preliminary data

主持人的技術

- 主持人具有相關技術的Publications
- 具有相關技術的共同或協同主持人

計畫共同主持人

協助計畫的順利進行

- 支援實驗所需要的技術
- 資深且學術有聲望的來協助
- 不要找太多共同主持人，有獨立性不夠之嫌

主持人的研究表現

- 論文發表情形
- 計畫的執行率

論文發表情形

- 論文發表的質與量
- 研究主題的連貫性
- 研究的獨立性

論文發表的質與量

- 對年輕學者的審查不會太過對文章質的要求，但有連貫性主題的文章會有加分效果
- 對資深學者文章的質與量都會要求

論文主題的連貫性

- 對年輕學者的審查不會太過對文章主題連貫性的要求，但有高品質的文章會有加分效果
- 對資深學者文章主題的連貫性會要求
- 如果要變換研究的主題，最好有充足的Rationale、Preliminary data、及Some related publications

研究的獨立性

- 對年輕學者的審查不會太過苛責是否有通訊作者文章，如有會有加分效果
- 對資深學者會要求為文章的通訊作者
- 不要有太多的共同第一或通訊作者
(過多文章為共同通訊作者不是好現象)

計畫的執行率

過去補助計畫的成果

- 論文發表
- 專利

研究計畫補助主題的選擇

- 時代熱門議題
- 各領域關注的議題
- 突破性的議題

主持人時間分配

主持人如果有兩個以上計畫或同時擔任主持人與共同主持人，要注意每週平均投入工作時數比率(%)的總和。同時要注意，除了研究還有教學的工作時數。

抄襲問題

- 每個計劃會先進行抄襲比對
- 如果是多年期計畫只過1年，隔年再提出申請，得註明是延續計畫，才不會發生自我抄襲的問題

計畫頁數問題

- 不予以審查
- 超過部分不再審查

計畫常見的問題

- 題目與內容不符
- 沒有假說(hypothesis)
- 沒有實驗設計
- 沒有preliminary data