科技部補助專題研究計畫成果報告
期末報告

第二型糖尿病相關因子對前列腺癌雄性素接受器訊息傳遞的調控

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 102-2320-B-040-023-
執 行 期 間 ： 102年08月01日至104年07月31日
執 行 單 位 ： 中山醫學大學醫學系生理學科

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報 告 附 件 ： 出席國際會議研究心得報告及發表論文

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中 華 民 國 104 年 10 月 31 日

中 文 摘 要 ： 前列腺癌的危險因子包括年齡、激素、種族、家庭病史以及飲食，近年來，流行病學的證據指示糖尿病史與前列腺癌的發生率有相反關係，但是流行病學家尚未得到肯定的一致性結果，因此糖尿病如何導致前列腺癌發生率的下降，此機制尚未全面瞭解。本計畫的研究目的是探討糖尿病的相關因子，包括葡萄糖、胰島素和和類胰島素生長因子 (IGF-1)對於前列腺癌細胞在離體狀況下增生的影響。細胞增生檢測以及激素接受器的表現分別利用MTT和西方墨點染色觀察，結果顯示糖尿病相關因子不會影響雄性素依賴性前列腺癌細胞的增生，然而對於非雄性素依賴性前列腺癌，則會造成細胞增殖的作用。在前列腺組織晶片的染色結果，癌化組織中雄性素接受器以及細胞核內的IGF-1表現強度皆比正常前列腺組織明顯。關於激素接受器的研究結果，胰島素以及IGF-1對於培養在低濃度血清培養液中的雄性素依賴性前列腺癌細胞LNCaP而言，胰島素接受器貝塔(IRβ)表現會下降，而類胰島素生長因子接受器貝塔 (IGF-1Rβ)則會增加，其他幾種前列腺癌細胞則無此結果。大部分的抗糖尿病用藥對於前列腺癌細胞具有促進生長的效果；但每福敏(metformin)則特別抑制會表現雄性素接受器的前列腺癌細胞生長。這些結果推論糖尿病相關因子可能藉由改變前列腺癌細胞中雄性素接受器、胰島素接受器和類胰島素生長因子接受器的表現而增加癌細胞的增生；然而每福敏則可能藉由調控前列腺癌細胞中雄性素接受器的訊息傳遞而達到抑制癌細胞增生的效果。

中 文 關 鍵 詞 ： 抗糖尿病藥物、糖尿病、類胰島素生長因子1、胰島素、前列腺癌

英 文 摘 要 ： Risk factors for prostate cancer (PCa) include age, hormones, race, family history and diet. Recently, epidemiologic evidence has indicated that history of diabetes mellitus (DM) is inversely associated with risk of PCa. However, epidemiological investigations have yielded inconsistent results. Hence, the exact mechanism of DM-induced reduction in the incidence of PCa has yet to be fully elucidated. The aim of this study was to investigate the effects of DM factors, including glucose, insulin and insulin-like growth factor-1 (IGF-1), on the proliferation of PCa cell lines in vitro. Cell proliferation and expression of hormone receptors was examined in MTT assay and western blot analysis, respectively. The results showed that DM factors did not affect the viability of androgen-dependent PCa cell lines. However, cell proliferation increased after treatment with DM factors in androgen-independent PCa cell lines. On PCa tissue arrays, intensities of total androgen receptor (AR) and nuclear IGF-1R were higher in malignant tissues than in normal prostate glands. In terms of hormonal receptors, androgen-dependent LNCaP cells treated with insulin and IGF-1 in a low-serum medium showed decreased expression of insulin receptor beta (IRβ) and elevated expression of IGF-1 receptor beta (IGF-1Rβ). Moreover, expression of AR was

upregulated after insulin and IGF-1 treatment in LNCaP cells, but not in the other PCa cell lines. Most of the studied antidiabetic drugs promoted the viability of PCa cells. However, metformin decreased the viability of AR-expressing PCa cells. These results suggest that diabetic factors modify the expression of AR, IR and IGF-1R to increase cancer cell proliferation. Moreover, the growth suppressing effects of metformin on PCa may be via the regulation of the AR signaling pathway.

英文關鍵詞：antidiabetic drugs, diabetes mellitus, IGF-1, insulin, prostate cancer

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

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計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST －102－2320－ B － 040 － 023
執行期間： 102 年 8 月 1 日至 104 年 7 月 31 日

執行機構及系所：中山醫學大學醫學系生理學科

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■出席國際學術會議心得報告

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中　華　民　國 104 年 10 月 31 日

目　　錄

中文摘要

前列腺癌的危險因子包括年齡、激素、種族、家庭病史以及飲食，近年來，流行病學的證據指示糖尿病史與前列腺癌的發生率有相反關係，但是流行病學家尚未得到肯定的一致性結果，因此糖尿病如何導致前列腺癌發生率的下降，此機制尚未全面瞭解。本計畫的研究目的是探討糖尿病的相關因子，包括葡萄糖、胰島素和和類胰島素生長因子 (IGF-1)對於前列腺癌細胞在離體狀況下增生的影響。細胞增生檢測以及激素接受器的表現分別利用 MTT 和西方墨點染色觀察，結果顯示糖尿病相關因子不會影響雄性素依賴性前列腺癌細胞的增生，然而對於非雄性素依賴性前列腺癌，則會造成細胞增殖的作用。在前列腺組織晶片的染色結果，癌化組織中雄性素接受器以及細胞核內的 IGF-1 表現強度皆比正常前列腺組織明顯。關於激素接受器的研究結果，胰島素以及 IGF-1 對於培養在低濃度血清培養液中的雄性素依賴性前列腺癌細胞 LNCaP 而言，胰島素接受器貝塔 (IRβ)表現會下降，而類胰島素生長因子接受器貝塔 (IGF-1Rβ)則會增加，其他幾種前列腺癌細胞則無此結果。大部分的抗糖尿病用藥對於前列腺癌細胞具有促進生長的效果；但每福敏 (metformin)則特別抑制會表現雄性素接受器的前列腺癌細胞生長。這些結果推論糖尿病相關因子可能藉由改變前列腺癌細胞中雄性素接受器、胰島素接受器和類胰島素生長因子接受器的表現而增加癌細胞的增生；然而每福敏則可能藉由調控前列腺癌細胞中雄性素接受器的訊息傳遞而達到抑制癌細胞增生的效果。

關鍵字：抗糖尿病藥物、糖尿病、類胰島素生長因子 1、胰島素、前列腺癌

英文摘要

Risk factors for prostate cancer (PCa) include age, hormones, race, family history and diet. Recently, epidemiologic evidence has indicated that history of diabetes mellitus (DM) is inversely associated with risk of PCa. However, epidemiological investigations have yielded inconsistent results. Hence, the exact mechanism of DM-induced reduction in the incidence of PCa has yet to be fully elucidated. The aim of this study was to investigate the effects of DM factors, including glucose, insulin and insulin-like growth factor-1 (IGF-1), on the proliferation of PCa cell lines *in vitro*. Cell proliferation and expression of hormone receptors was examined in MTT assay and western blot analysis, respectively. The results showed that DM factors did not affect the viability of androgen-dependent PCa cell lines. However, cell proliferation increased after treatment with DM factors in androgen-independent PCa cell lines. On PCa tissue arrays, intensities of total androgen receptor (AR) and nuclear IGF-1R were higher in malignant tissues than in normal prostate glands. In terms of hormonal receptors, androgen-dependent LNCaP cells treated with insulin and IGF-1 in a low-serum medium showed decreased expression of insulin receptor beta (IRβ) and elevated expression of IGF-1 receptor beta (IGF-1Rβ). Moreover, expression of AR was upregulated after insulin and IGF-1 treatment in LNCaP cells, but not in the other PCa cell lines. Most of the studied antidiabetic drugs promoted the viability of PCa cells. However, metformin decreased the viability of AR-expressing PCa cells. These results suggest that diabetic factors modify the expression of AR, IR and IGF-1R to increase cancer cell proliferation. Moreover, the growth suppressing effects of metformin on PCa may be *via* the regulation of the AR signaling pathway.

Key Words: antidiabetic drugs, diabetes mellitus, IGF-1, insulin, prostate cancer

前　　言

Incidences of prostate cancer (PCa) have been high in recent decades, and it is the second-leading cause of cancer death among men in the US (11). A total of 220,800 new cases of PCa and 27,540 deaths due to PCa are estimated in the US in 2015 (28). According to statistical data from the Ministry of Health and Welfare, Taiwan, PCa mortality has increased from 7.1/100,000 persons to 10.4/100,000 persons in the past decade. PCa is currently the fifth-leading cause of cancer death, up from the seventh-leading cause, in Taiwan. Prostate-specific antigen (PSA) assay has allowed early detection of PCa, which is curable by surgical or radiation therapies. Androgen ablation is the major treatment for metastatic PCa. However, 20% to 30% of patients with PCa endure recurrent disease (8, 12). Therefore, chemoprevention and chemical control of PCa have become major concerns.

Both PCa and diabetes mellitus (DM) are common in elderly men. Previous epidemiologic studies have found that patients with diabetes mellitus type 2 (DM2) have lower risk of PCa (10% to 40% lower than subjects without DM) (2, 16). This finding is consistent across various ethnic populations in the US (25), such as European-American, African-American, Native Hawaiian, and Japanese-American (41), as well as a Taiwanese population (30, 33). There are several possible theories for this phenomenon: (1) The plasma hormone levels of testosterone, insulin and insulin-like growth factor-1 (IGF-1) are lower in patients with diabetes mellitus type 1 (DM1) and uncontrolled DM2. Lower hormone levels may have prohibited PCa cell growth (16). (2) The level of PSA, a marker of androgenic signaling, is lower in DM patients than in subjects without diabetes (22). (3) Antidiabetic drugs have an inhibitory effect on PCa (7). (4) Different stages of DM have different effects on PCa. For example, newly diagnosed DM patients have higher incidence of PCa. However, as DM progresses, PCa risk decreases (25). (5) Patients with DM may seek medical help and, in the process, PCa may be diagnosed (17). (6) In comparison with the

lowest quartile of DM2 risk allele count, the highest quartile is negatively correlated with PCa risk (24).

In contrast, a meta-analysis of Asian populations has indicated that diabetes is positively correlated with the risk of PCa (20). A series of research articles by Tseng *et al.* has suggested that the prevalence (15), incidence (33) and mortality (37) of PCa increase in DM patients. Therefore, population differences might affect the relationship between DM and risk of PCa.

Clinical information provides too many variables for determination of the exact mechanism and relationship between PCa progression and DM in DM patients. In the present study, diabetic factors and antidiabetic drugs were analyzed to determine their associations with possible expression viability of and hormone receptors in PCa cell lines. It is anticipated that the results of this study will provide valuable information for clinical treatment and management of PCa.

研　究　方　法

Cells and Culture Conditions

Androgen-dependent prostate cancer cell lines, LNCaP and 22RV1, and androgen-independent prostate cancer cell lines, PC3 and DU145, were purchased from the Culture Collection and Research Center (CCRC) of the Food Industry Research and Development Institute (FIRDI), Taiwan, R.O.C. Cell lines were maintained in RPMI 1640 (Gibco Laboratories, Buffalo, NY, USA) (LNCaP and 22RV1), in minimum essential medium (Gibco)(DU145) or Kaighn's modification of F-12K Ham medium (Sigma, St. Louis, MO, USA)(PC3), with 50 IU/ml potassium penicillin G (Sigma), 50 IU/ml streptomycin sulfate (Sigma) and 10% fetal bovine serum (Sigma) in an atmosphere of 5% CO_2 at 37 ℃.

Cell Viability Assessment

The modified colorimetric 3-(4,5-dimethylthiazol-2-yle) 2,5-diphenyltetrazolium bromide (MTT) (Sigma) assay was employed to quantify cell viability. Briefly, cells were incubated on 96-well microplates (Falcon, Franklin Lakes, NJ, USA) for 24 h at a density of 2,000 cells/well during the pre-incubation process. The culture medium was then removed and replaced with medium containing 1% fetal calf serum (FCS) alone, or with various concentrations of insulin (10, 50, 100 nM) (Sigma), IGF-1 (10, 50, 100 ng/ml) (Sigma) or glucose (1, 5, 10 mM) (Sigma). To determine the anti-proliferative effects of antidiabetic drugs on the PCa cell lines, culture media were replaced with glibenclamide (Sigma), acarbose (Sigma), pioglitazone (Sigma), or repaglinide (Sigma) at a concentration of 0.01 to 10 μM. Metformin (Sigma) was used at a concentration of 1 to 10 mM. Following treatment for 48 h, culture media were removed and replaced with 50 μl 1 mg/ml MTT solution in serum-free medium. After an additional 4-h incubation period, MTT solution was replaced with 50 μl DMSO, and the plates were shaken for 3 min. The optical density of each well was

determined using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm with a reference wavelength of 630 nm. Each experimental condition was repeated 3 times.

Immunoblotting Assessment

After culture in the presence of insulin, IGF-1 and glucose for 24 h in 1% FCS medium, cells were lysed in RIPA buffer (50 mM Tri-HCl, pH 7.4, 1 % NP-40, 0.25 % Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, l mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM Na-orthovanadate, 1 mM NaF) on ice for 30 min. Cell lysate was centrifuged at 10,000 ×*g* at 4 ℃ for 15 min, and the supernatant was collected. Equal amounts of cell extract proteins (50 μg) were subjected to 12% SDS-PAGE and transferred to PVDF membranes (PerkinElmer, Waltham, MA, USA). Membranes were incubated in blocking solution (5% dry milk in TBST containing 20 mM Tris-HCl, 135 mM NaCl, 0.1 % Tween 20, pH 7.6), followed by incubation with a primary antibody overnight. The following primary antibodies were used at a concentration of 1 μg/ml: phosphor-Ser-81-androgen receptor (AR^{PSer81}) (Millipore, Billerica, MA, USA), insulin receptor β (IRβ) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), insulin-like growth factor-1 receptor β (IGF-1Rβ) (Santa Cruz) and β-actin (Sigma). After washing three times with TBST, the blot was incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody (1:5,000) (Santa Cruz), and proteins were visualized using enhanced chemiluminescence detection (ECL) (Perkin Elmer). Protein expression was detected by chemiluminescence/fluorescence imaging analyzer (Fujifilm, Tokyo, Japan). Each experimental condition was repeated three times.

Immunohistochemical Detection

Human prostate tissue arrays (Biomax, Rockville, MD, US) were purchased to detect protein expression in normal and cancerous tissues. Paraffin-embedded tissue arrays were deparaffinized and hydrated to visualize expression of AR, IR and IGF-1R. Antigen retrieval was done by incubating the sections in boiling 10 mM citrate buffer, pH 6.0, for 20 min. Endogenous peroxidase activity was inhibited by 3% (v/v) H_2O_2 in methanol. Sections were then incubated with rabbit polyclonal anti-AR, anti-IR or anti-IGF-1R antibodies (Santa Cruz) at 1:50 dilutions. Finally, the sections were stained with ultravision quanto detection system (Thermo Scientific, Pittsburgh, PA, USA) according to the manufacturer's instructions. Graded alcohol and xylene were applied to the sections, which were then coverslipped with a mounting medium. Sections were observed at 40x or 100x magnification.

Statistical Analyses

All values are presented as the mean ± standard error of the mean (SEM). Means were tested for homogeneity by one-way analysis of variance (ANOVA), and the difference between specific means was tested for significance by Duncan's multiple-range test. The difference between two means was considered statistically significant when $P < 0.05$.

結　　果

Pro-Proliferative and Anti-Proliferative Effects of Insulin, IGF-1 and Glucose on Human PCa Cells

As DM2 presents higher levels of blood glucose in combination with higher insulin and IGF-1 levels, the viabilities of both androgen-dependent and -independent PCa cell lines were examined after treatment with insulin, IGF-1 and glucose (Fig. 1). Treatment concentrations used in this study were based on those of previous DM studies (21, 27, 45). Cell viability was examined in MTT assays. In LNCaP cells, insulin and glucose did not show significant growth-promoting effects. IGF-1 at a concentration of 100 ng/ml elevated the viability in comparison to the control group. In 22RV1 cells, none of the 3 factors revealed significant changes in terms of viability. All 3 factors exhibited pro-proliferative effects on DU145 cells. Viability of PC3 cells increased only after treatment at the highest concentration.

Effects of Modulation of Protein Expression of Hormone Receptors by Insulin, IGF-1, and Glucose

Previous studies have found that in addition to AR, other hormone receptors have growth promoting or inhibitory effects on PCa. In the present study, AR, and the insulin-related receptors, IRβ and IGF-1Rβ, were examined on tissue arrays of normal and malignant prostate sections. Representative images are shown in Fig. 2. From the results of H&E staining (Figs. 2A to 2D), malignant tissues were less differentiated and had higher nuclear-cytoplasmic ratio in comparison to normal prostate. Total AR (Figs. 2E to 2H) was expressed in both the cytoplasm and the nucleus of normal prostate gland and malignant tissue. The intensity of AR staining was stronger in stage IV prostate cancer tissues. IRβ (Figs. 2M to 2P) was expressed in the cytoplasm of normal prostate gland cells. However, expression was lower in cancerous tissues. In addition, expression of IGF-1Rβ (Figs. 2I to 2L) was primarily in the cell membrane

and cytoplasm of the stroma in normal prostate tissues. This phenomenon was previously reported to be observed in rapidly proliferating non-malignant cells (1). There was minor nuclear staining in gland cells. Significantly higher nuclear staining of IGF-1R was observed in stage IV adenocarcinoma (Figs. 2K and 2L).

AR, IRβ and IGF-1Rβ were expressed in malignant PCa tissues. Thus, these hormone receptors were further investigated in the PCa cell lines (Fig. 3). Cells were divided into five groups: control (10% FCS medium), 1%S (1% FCS medium), Ins (100 nM insulin), IGF-1 (100 ng/ml IGF-1) and Glc (10 mM glucose). Insulin, IGF-1 and glucose used in this study were prepared in 1% FCS medium. After incubation in 1% FCS medium, AR phosphorylation at serine 81 (AR^{PSer81}) decreased in LNCaP and 22RV1 cells. Insulin, IGF-1 and glucose treatments led to the recovery of AR^{PSer81} expression in LNCaP cells. Moreover, insulin and IGF-1 decreased IR expression and increased IGF-1R expression in comparison to the 1% FCS group (Fig. 3A). However, these phenomena were not observed in AR-expressing 22RV1 cells. In 22RV1 cells, insulin and IGF-1 had no effects on AR^{PSer81} expression, but insulin downregulated the expression of IRβ and IGF-1Rβ. Similar results were found in two other androgen-independent PCa cell lines, DU145 and PC3 (Fig. 3B).

Promoting and Inhibitory Effects of Antidiabetic Drugs on the Viability of PCa Cell Lines

One of the theories about the inverse correlation of DM history and PCa risk is that antidiabetic drugs inhibit PCa growth (7). The categories of antidiabetic drugs used in this study included sulfonylurea (glibenclamide), megnitilide (repaglinide), biguanide (metformin), alpha-glucosidase inhibitor (acarbose) and thiazolidinedione (pioglitazone). The ranges of drug concentrations applied to the cells matched those of plasma dosages in DM patients. Glybenclamide, repaglinide, acarbose and pioglitazone showed pro-proliferative effects on both androgen-dependent (LNCaP

and 22RV1) and androgen-independent (DU145 and PC3) cells, with the most significant effects produced by pioglitazone and acarbose (Fig. 4). Metformin, the antidiabetic drug most often used for inhibiting glycogenolysis, revealed anti-proliferative effects on androgen-dependent PCa cell lines, especially on LNCaP cells (Fig. 5).

討　　論

In the present study, we demonstrated the following: (1) Insulin, IGF-1 and glucose do not significantly affect the viability of androgen-dependent PCa cells in comparison to control group (1% FCS medium) (Fig 1). (2) Insulin, IGF-1 and glucose have pro-proliferative effects on androgen-independent PCa cells (Fig. 1). (3) IGF-1Rβ is elevated in the nucleus of PCa tissues (Fig. 2). (4) Insulin and IGF-1 increase AR^{PSer81} expression in the LNCaP cell line but not in the other PCa cell lines (Fig. 3). (5) Both insulin and IGF-1 show inverse results for the expressions of IRβ versus IGF-1Rβ in LNCaP cells (Fig. 3). (6) IRβ and IGF-1Rβ expressions are downregulated in 22RV1, DU145, and PC3 cell lines after treatment with insulin and IGF-1 (Fig. 3). (7) Most of the antidiabetic drugs used in this study have pro-proliferative effects on PCa cells (Fig. 4). (8) Metformin exhibits anti-proliferative effect on androgen-dependent PCa cells, especially LNCaP cells (Fig. 5).

Under clinical conditions, standard treatments for prostate cancer are surgery and medical castration to depress the proliferative function of AR (5). Recurrence of PCa after androgen-deprivation therapy (ADT) is referred to as castration-resistant prostate cancer (CRPC). Most CRPCs demonstrate high AR expression. Approximately 30% of CRPC patients show AR gene amplification and 15% show an increase in AR co-activator (44). In our study, insulin revealed no effects on cell viability in androgen-dependent PCa cells, but increased proliferation of androgen-independent cells (Fig. 1). The androgen-independent cell lines, which responded to insulin stimulation, did not express AR or PSA. Insulin did not increase the proliferation of AR-expressing PCa cell lines. This may explain the lack of clinical effectiveness of insulin use on the risk and mortality of PCa (34, 35).

Insulin, IGF-1 and glucose generally promote cancer cell growth (21, 27). Epidemiologic studies have indicated that hyperinsulinemia and hyperglycemia are

related to increased risk of several cancers, such as melanoma and of the colon, pancreas, breast, lung, endometrium and liver (10, 13, 14, 40). In addition, high IGF-1 and low IGF-binding protein (IGFBP)-1 in plasma contribute to the occurrence of PCa. IGF-1R and IR may have the potential to form hybrid receptors which enhance tumorigenesis and tumor vascularization (29). Activated IGF-1R targets many signaling pathways, including Ras/Raf/MAPK, PI3K/Akt, STAT3 and Twist1, promoting cell growth and viability (31, 46). Due to different properties of the PCa cell lines used in this study, insulin, IGF-1 and glucose showed distinct growth-promoting effects after 48-h challenge (Fig. 1).

AR is important for the growth of PCa. Higher levels of total AR were found to be expressed in cancerous tissues than in normal prostate gland in the present study (Fig. 2). AR may be activated via ligand-dependent mechanism (*e.g.* testosterone or dihydrotestosterone) (4) or ligand-independent pathway (38). Activated AR (AR^{PSer81}) binds to androgen response element (ARE) and regulates expression of downstream genes, as well as influences PCa cell growth and PSA secretion (8). IGF-1R, IR and their downstream factors are involved in androgen-independent progression and malignancy of PCa (26). The relationship between AR and IGF-1R is one of two-way regulation. Dihydrotestosterone (DHT) not only stimulates the growth of CRPC**,** but also elevates the protein and mRNA levels of IGF-1R via a genomic pathway (26). In addition, androgen upregulates IGF-1R by phosphorylating cyclic AMP response element binding protein (CREB) binding protein (CBP), in AR wild-type and mutant PCa cells via a non-genomic pathway (9). Blocking the activation of IGF-1R downregulates androgen-mediated gene expression (43) and nuclear localization of AR. Moreover, IGF-1R and AR engage in crosstalk to activate the downstream factors that synchronically accelerate tumor growth in PCa.

Accumulation of nuclear IGF-1R (nIGF-1R) relates inversely to the survival of cancer patients, including those with renal cancer or rhabdomyosarcoma (1, 39). In

normal cells, there is little or no nIGF-1R expression. However, the ratio of nuclear/membrane IGF-1R is 13-fold higher in breast cancer cells than in breast epithelial cells (6). Over -accumulation of nIGF-1R may downregulate gene expression, contributing to the growth of cancer cells (6). In this work, IGF-1R expression was higher in cancerous cells than in normal prostate tissue, especially in the nucleus (Fig. 2K). Although, there are currently no published reports on the expression of nIGF-1R in PCa, the strong nuclear staining of IGF-1R in stage IV PCa tissues reveals a possible role for nIGF-1R in the pathophysiology of PCa.

AR^{PSer81} decreased after incubation in 1% FCS medium in AR-expressing PCa cell lines (Fig. 3). This might have resulted from lower levels of androgen in the medium (32). LNCaP cell line requires AR signaling for proliferation. AR^{PSer81} and IGF-1R expression was recovered to maintain cell growth after insulin or IGF-1 treatment in 1% FCS medium. IGF-1R elevates AR transcriptional activities, including AR phosphorylation and translocation to the nucleus (42). Thus, insulin and IGF-1 may increase AR^{PSer81} expression via IGF-1R signaling (Fig. 3A). Although IR and IGF-1R can be activated by the same ligand, IR and IGF-1R do not play equal roles. IR is more metabolic, whereas IGF-1R is more mitogenic. The ratio of these two receptors alters when cells encounter different environments (3). Therefore, IGF-1R showed higher expression than IR after insulin and IGF-1 treatment in LNCaP cells under low androgen conditions (Fig. 3A). In addition, IR expression was lower in malignant tissue than in normal prostate gland (Fig. 2M-2P). In the PCa cell lines, insulin downregulated IR expression in parallel with hyperinsulinemia, consistent with decreased IR in DM2 patients (23).

Meta-analyses have demonstrated an inverse correlation between DM and PCa risk. This may be the result of medical treatment of DM patients (6, 17). The classification of antidiabetic drugs includes insulin stimulator (sulfonylurea and megnitilide), glucose uptake stimulator (biguanide and thiazolidinedione), and glucosidase inhibitor.

One drug of each type was selected to challenge PCa cell lines in our work. As glucose can be used as a fuel for cancer cells, these medications either directly facilitate the utilization of glucose, or indirectly promote cancer cell growth by increasing insulin concentration. Not surprisingly, the five drugs used in this study showed pro-proliferative effects on the androgen-independent PCa cell lines DU145 and PC3 (Fig. 4). However, a single drug from each class cannot be considered representative of the function of all the drugs in that class. Our data indicated the effects of only these five drugs on the proliferation of PCa cells.

In androgen-dependent PCa cells, only metformin revealed anti-growth effects at higher concentrations (Fig. 5). Metformin exert impacts on mitochondrial respiration and activates AMP-activated protein kinase (AMPK), which modulates energy homeostasis of cells. AMPK also reduces gluconeogenesis, which results in decline in insulin and its downstream pathway (19). Hence, metformin has an anti-growth or even pro-apoptotic effect on cancer cells. In addition, metformin inhibits AR functions leading to growth suppression of AR-expressing PCa cells (18). This may have resulted in the specific inhibitory effect of metformin on androgen-dependent PCa cell lines in this study (Fig. 5).

There is currently no precise treatment for DM2 in patients with cancer. Our data have indicated that most antidiabetic medications and plasma factors of DM2 patients, such as insulin, IGF-1 and glucose, are associated with PCa tumor growth. Alterations of the expression of AR, IR and IGF-1R are involved in tumor progression. However, metformin suppressed cell viability especially in AR-expressing PCa cells. These findings revealed that it is not DM2, but rather the medication used to treat it, that has the potential for PCa treatment. Future studies are needed to translate the application of these findings to clinical management of PCa patients with DM2.

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Figure 1

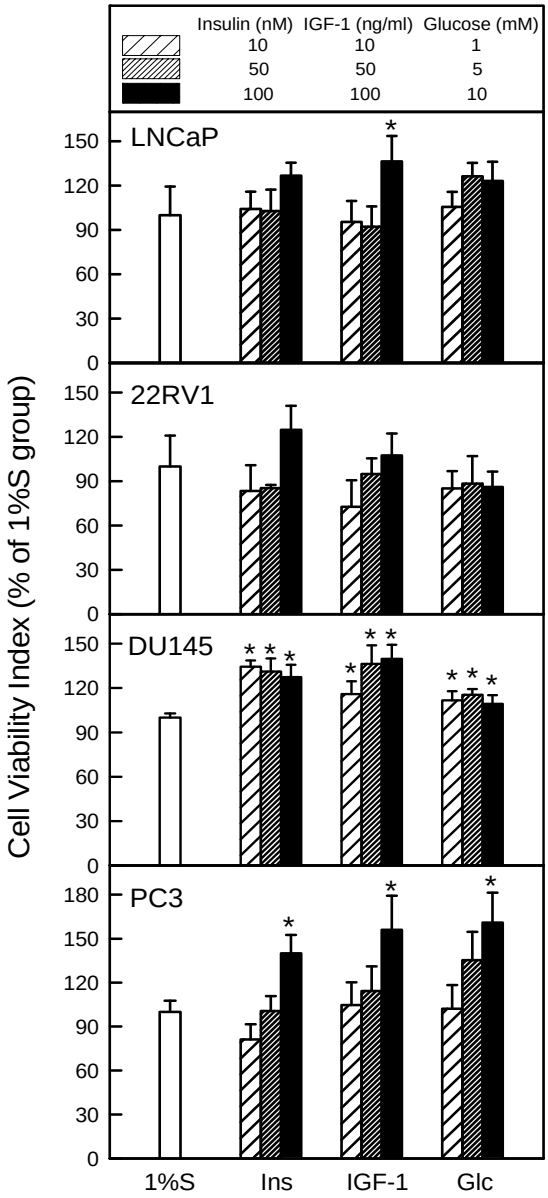

Fig. 1. Effects of insulin, IGF-1 and glucose on the viabilities of PCa cell lines. Cells were cultured in 1% FCS medium (1%S) and then challenged with insulin, glucose or IGF-1 for 48 h. The levels of insulin (Ins), IGF-1 and glucose (Glc) were 10, 50, 100 nM; 10, 50, 100 ng/ml and 1, 5 10 mM, respectively. Cell viability was measured in MTT assays. Each value represents mean ± SEM. Control value (1%S group) = 100%; **P* < 0.05 versus 1%S group.

Figure 2

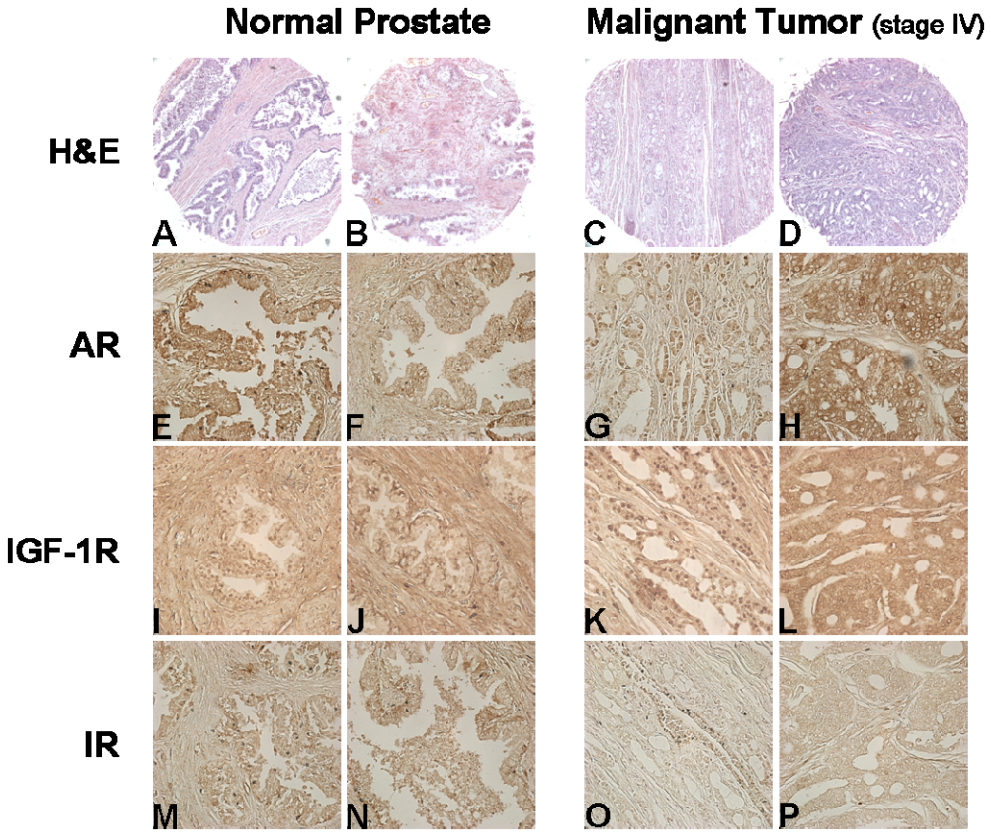

Fig. 2. Protein expression levels of AR, IGF-1R and IR in normal and malignant human prostate tissues. Tissue arrays were examined with H&E stain and IHC stain as described in Materials and Methods. A - D: H&E staining observed at 40x magnification; E - H: IHC of AR; I - L: IHC of IGF-1R, and M - P: IHC staining of IR observed at 400x magnification.

Figure 3

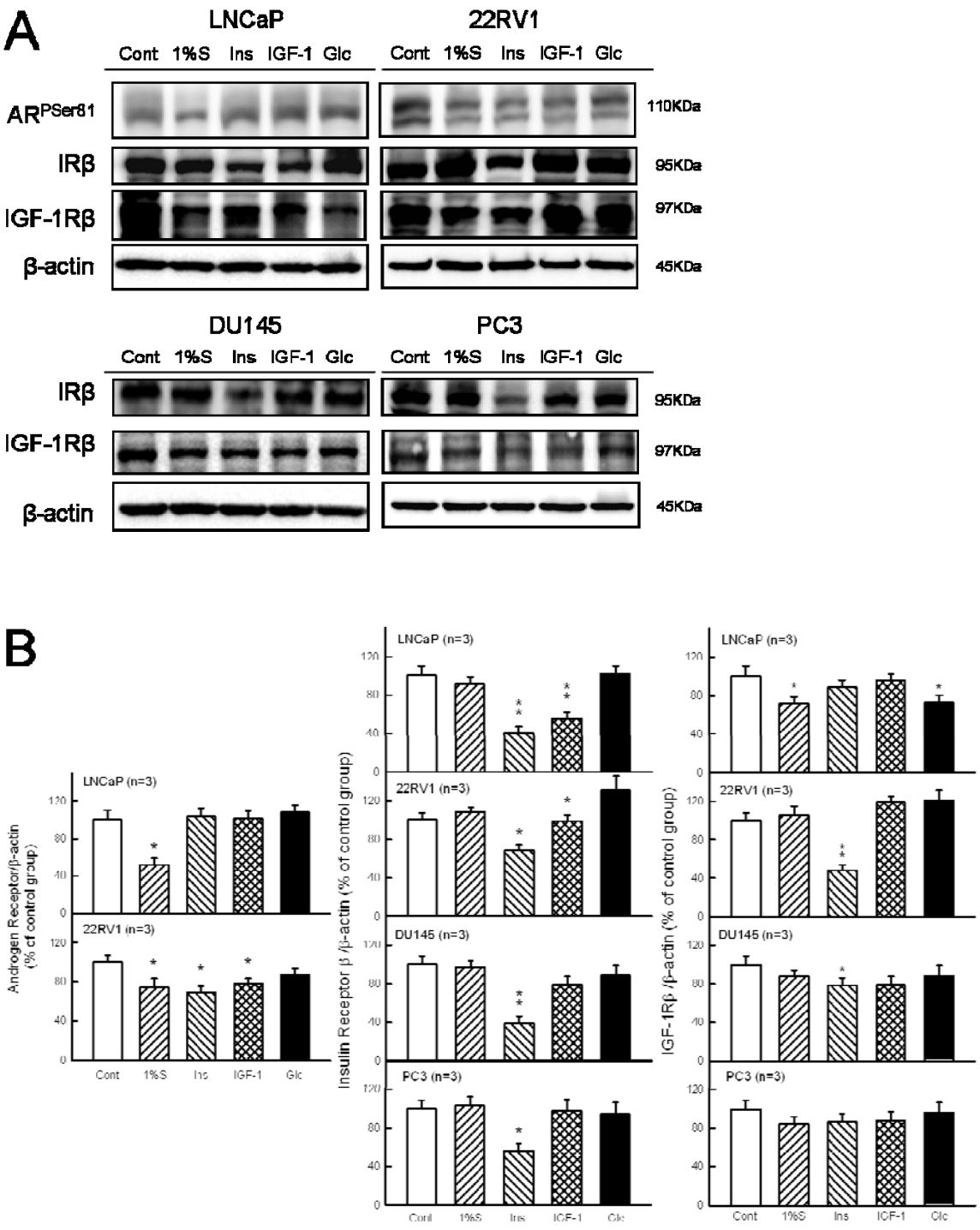

Fig. 3. Protein expression levels of AR^{PSer81}, IRβ and IGF-1Rβ after treatment with insulin, IGF-1 and glucose in both AR-expressing and non-AR-expressing PCa cell lines. Cells were incubated with insulin (100 nM), IGF-1 (100 ng/ml) or glucose (10

mM) for 24 h. Whole-cell lysates were subjected to 8% SDS-PAGE. Each lane was loaded with 50 μg protein. Similar results were obtained from three other experiments. B. Quantitative data of protein expression including AR^{PSer81}, IRβ, or IGF-1Rβ. $P < 0.05$. Each value represents mean ± SEM. Cont: control; 1%S: medium with 1% FCS; Ins: insulin; Glc: glucose.

Figure 4

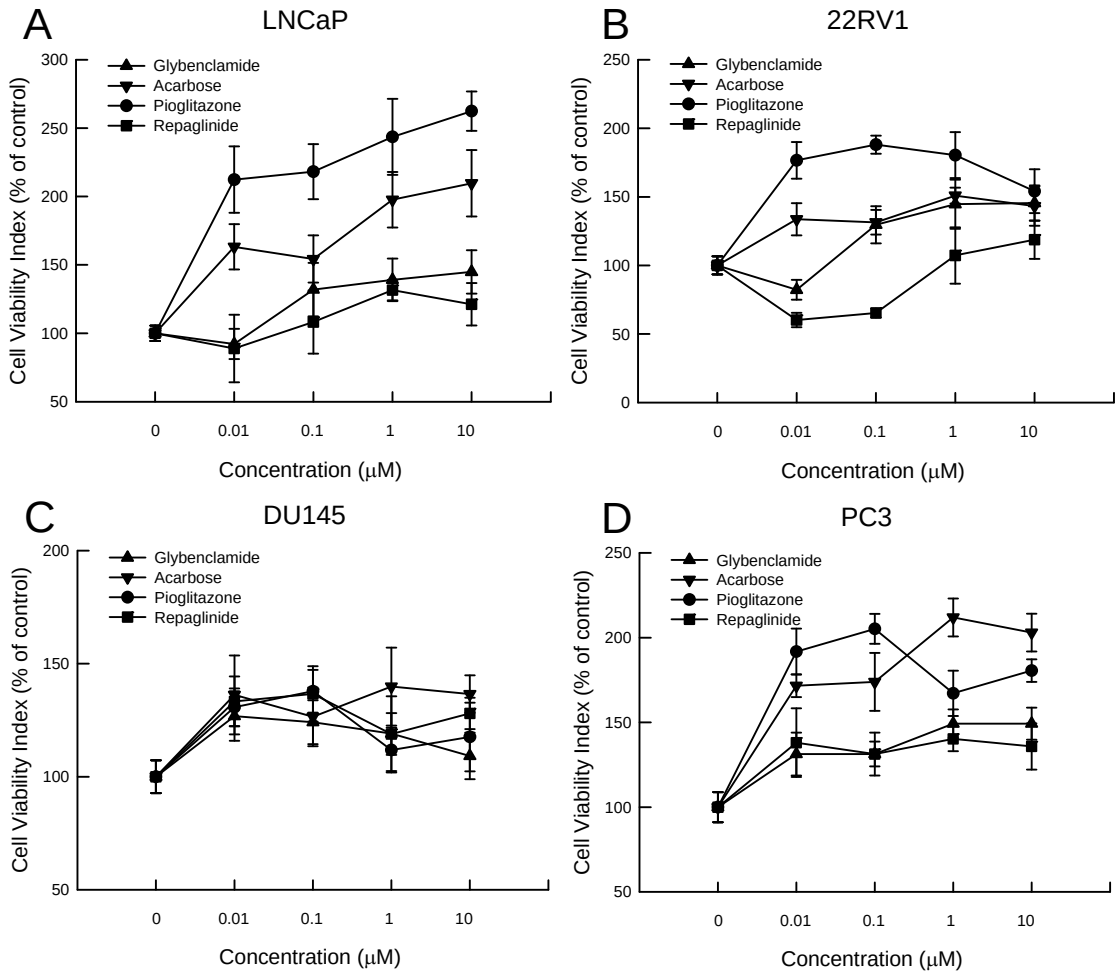

Fig. 4. Viabilities of PCa cell lines following treatment with antidiabetic drugs. Following challenges at concentrations of 0.01, 0.1, 1 and 10 μM for 48 h, the cell proliferation relative to the day 0 group was measured in MTT assay. Each value represents mean ± SEM.

Figure 5

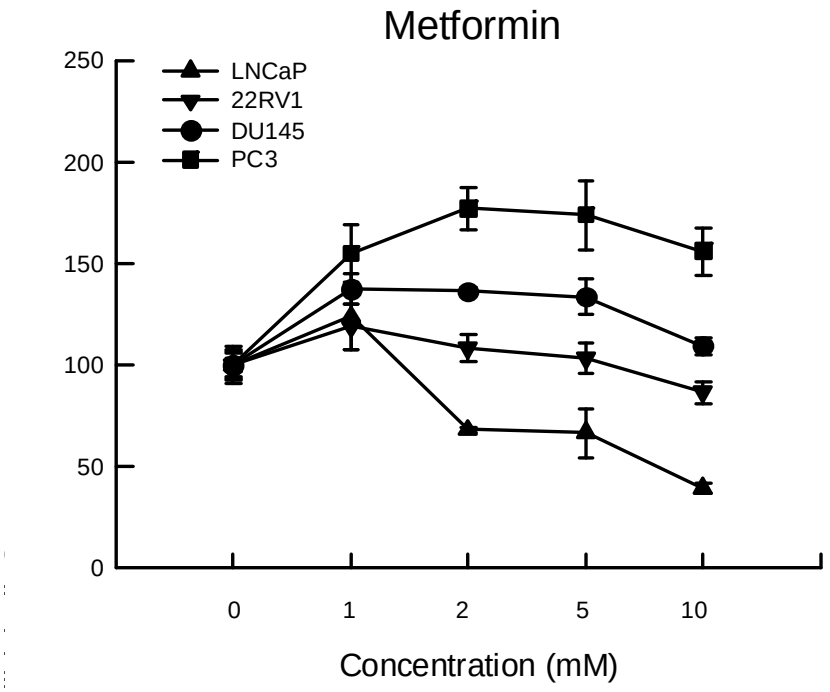

Fig. 5. Viabilities of PCa cell lines following metformin treatment. Following challenges at concentrations of 1, 2, 5 and 10 mM for 48 h, the cell proliferation relative to the day 0 group was measured in MTT assay. Each value represents mean ± SEM.

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否有嚴重損及公共利益之發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□ 未達成目標（請說明，以 100 字為限）

□ 實驗失敗

□ 因故實驗中斷

□ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 □無

其他：（以 100 字為限）

研究成果已發表一篇論文（已被 Chinese Journal of Physiology 被接受但尚未出刊，接受函如附），另一篇為尚未發表之文稿。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性），如已有嚴重損及公共利益之發現，請簡述可能損及之相關程度（以 500 字為限）

在台灣的流行病學研究中，metformin 的使用可減少前列腺癌的發生率，另外，胰島素的使用並不會增加前列腺癌的罹患率，關於以上兩點，本研究計畫的結果，皆可為臨床流行病學研究作為基礎研究的驗證。再者，本計畫更進一步發現胰島素對於不表現雄性素接受器前列腺癌細胞生長有促進效果（表現雄性素接受器前列腺癌細胞之生長則無），臨床結果顯示，多數前列腺癌患者仍然表現雄性素接受器或相關活化蛋白，可以解釋胰島素不影響前列腺癌罹患率的原因。因此本研究可解釋流行病學的結果，並且希望可應用在臨床的診斷與治療。

Date: Mon, 24 Aug 2015 15:36:01 +0800
From: "The Chinese Journal of Physiology (CJP)" <cjp.editorial.office@gmail.com>
To: chyu <chyu@csmu.edu.tw>
Subject: CJP#104368 is Accepted for Publication in CJP

中國生理學雜誌

The Chinese Journal of Physiology

August 24, 2015

Dr. Ching-Han Yu
Department of Physiology
School of Medicine
Chung Shan Medical University
Taichung, Taiwan, ROC

Dear Dr. Yu,

Now I am so pleased to inform you that your manuscript (**CJP#104368**) entitled "Growth Modulation of Diabetes Factors and Antidiabetic Drugs on Prostate Cancer Cell Lines" (by Shiaw-Wen Chien, Dong-Yih Kuo, Jiuan-Miaw Liao, Paulus S. Wang and Ching-Han Yu) is accepted for the publication in ***The Chinese Journal of Physiology*** (CJP).

Many thanks to you for this outstanding work with which you greatly honor CJP.

Best regards,

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科技部補助專題研究計畫出席國際學術會議心得報告

日期： 104 年 10 月 31 日

計畫編號	MOST －102－2320－ B － 040 － 023		
計畫名稱	第二型糖尿病相關因子對前列腺癌雄性素接受器訊息傳遞的調控		
出國人員姓名	余青翰	服務機構及職稱	中山醫學大學醫學系生理學科助理教授
會議時間	104 年 2 月 26 日 至 104 年 2 月 27 日	會議地點	新加坡
會議名稱	(中文)2015 幹細胞臨床應用會議 (英文)2015 Clinical Applications of Stem Cells		
發表題目	(中文)每福敏對前列腺癌細胞幹細胞特性之調控 (英文) Alteration of Stemness Properties of Prostate Cancer Cells by Metformin		

一、參加會議經過

幹細胞臨床應用 (Clinical Applications of Stem Cells)相關研究已經進行很長一段時間，並且在世界各地也舉辦相關會議，2014 年新加坡的一群學者邀請多國講者在新加坡醫院國際會議中心舉辦次亞洲的幹細胞臨床應用會議，與會學者多來自新加坡本地，但也邀請了英國、美國、日本、印度和以色列的講者前來分享他們的研究成果，並且有來自韓國的學者進行海報發表，與會成員多元豐富，研究成果新穎值得探究。

二、與會心得

這次會議舉辦的地點在新加坡，正值新加坡的新年與建國 50 週年慶典，可藉此機會吸引外國學者與會，並且宣揚新加坡國慶。這個國家地理位置方便抵達，雖然天氣較為炎熱，但不易下雨出入行動方便，再加上便捷的大眾運輸工具和中文與英文皆可溝通的優勢，有利於舉辦國際會議。該國家的薪資約台灣的兩倍，因此註冊費和旅館住宿費用皆較台灣來得高，有意願至新加坡參與會議者需有心理準備。論文海報展示區、廠商展示區、電腦網路使用區以及飲料

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三、發表論文全文或摘要

Risk factors of PCa are including genetic factors, racial, hormones, and diet. After the conventional treatment, the recurrent PCa shows higher cancer stem cell properties than the original cell. Epidemiologic evidence indicates that the history of type 2 diabetes mellitus (T2DM) decreases the risk of PCa. The possible reason might be the drug used for DM, such as metformin. Only few studies examine the mechanism of anti-stem cell properties of metformin. In the present study, inhibitory effect of metformin on stemness properties expression in PCa was investigated. Androgen-dependent and -independent PCa cell lines were divided into five group, including control and metformin treatment groups. Wound healing assay and transwell assay were used to determine the migration and invasion abilities of PCa. Self renewing property was examined by colony formation assay. Protein expressions of stem cell-related protein were analyzed by Western blot. Metformin inhibited the cell migration of both cell lines after 24-hour of treatment. Downregulation of cell invasion and colony formation by metformin were also significantly. Expression of stem cell-related protein, Oct4, was decreased in 22RV1 cells after metformin treatment. These results might suggest that metformin might suppress PCa growth via inhibiting the survival of stem cell population. Type 2 diabetes mellitus shows inverted relationship with PCa risk. Antidiabetic medication metformin might contribute to this relativity. Metformin might inhibit PCa risk by decreasing stemness properties including cell migration, invasion, self-renewing and protein marker expression.

四、建議

出席國際會議的旅程，一路上收穫良多，在語言、知識、舉辦國際會議等都是很好的經驗，可以得到許多與國外學者交流，甚至合作的機會，也替自己未來的學術之路鋪下較廣的人脈。參與國際研討會，不止讓我們本身的研究可以被外國學者所看到，而給予寶貴之意見，減少在研究上走冤枉路，並且讓思考邏輯方面，得以更完整而深入。更重要的是，出國聽聽其他先進數年甚至數十年的研究，可以得知這些學者們的整個研究歷程，對於自身未來的研究更有啟發。對於鼓勵年輕學者與會的作法十分認同，這樣可以為該會議種下種子，未來才有繼續蓬勃發展的可能，所以舉辦國際會議，能利用 Travel award 以及註冊費和住宿費優待的方式，吸引年輕學者的與會。

五、攜回資料名稱及內容

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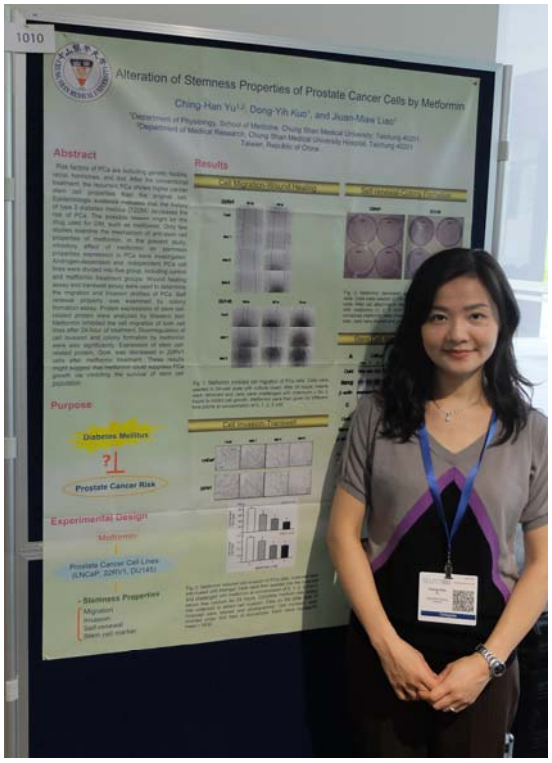

科技部補助專題研究計畫出席國際學術會議心得報告

日期： 104 年 10 月 31 日

計畫編號	MOST －102－2320－ B － 040 － 023		
計畫名稱	第二型糖尿病相關因子對前列腺癌雄性素接受器訊息傳遞的調控		
出國人員姓名	余青翰	服務機構及職稱	中山醫學大學醫學系生理學科助理教授
會議時間	104 年 2 月 26 日 至 104 年 2 月 27 日	會議地點	新加坡
會議名稱	(中文)2015 幹細胞臨床應用會議 (英文)2015 Clinical Applications of Stem Cells		
發表題目	(中文)每福敏對前列腺癌細胞幹細胞特性之調控 (英文) Alteration of Stemness Properties of Prostate Cancer Cells by Metformin		

一、參加會議經過

幹細胞臨床應用 (Clinical Applications of Stem Cells)相關研究已經進行很長一段時間，並且在世界各地也舉辦相關會議，2014 年新加坡的一群學者邀請多國講者在新加坡醫院國際會議中心舉辦次亞洲的幹細胞臨床應用會議，與會學者多來自新加坡本地，但也邀請了英國、美國、日本、印度和以色列的講者前來分享他們的研究成果，並且有來自韓國的學者進行海報發表，與會成員多元豐富，研究成果新穎值得探究。

二、與會心得

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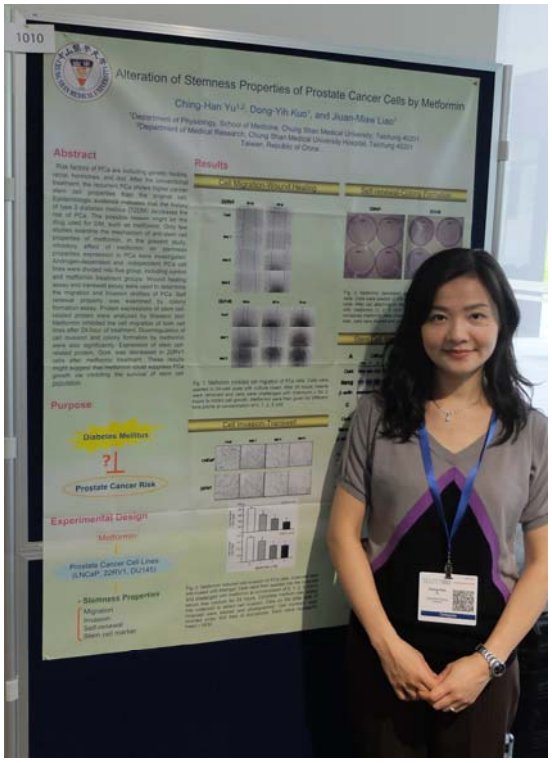

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/31

科技部補助計畫	計畫名稱: 第二型糖尿病相關因子對前列腺癌雄性素接受器訊息傳遞的調控
	計畫主持人: 余青翰
	計畫編號: 102-2320-B-040-023- 學門領域: 生理

無研發成果推廣資料

102年度專題研究計畫研究成果彙整表

計畫主持人：余青翰	**計畫編號：**102-2320-B-040-023-
計畫名稱：第二型糖尿病相關因子對前列腺癌雄性素接受器訊息傳遞的調控	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	4	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	0	50%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	80%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）	由於所屬之生理科沒有研究所，參與計畫之人力共有七位大學生，由於參與此計畫之研究，其中六位大學生對於研究皆產生濃厚興趣，目前已有四位就讀研究所（其中兩位有意願直升博士班繼續深造），另外兩位正參加推薦甄選希望進入碩士班就讀。因此此計畫已激發興趣，為台灣培養研究人才。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形：
   論文：■已發表 □未發表之文稿 □撰寫中 □無
   專利：□已獲得 □申請中 ■無
   技轉：□已技轉 □洽談中 ■無
   其他：（以100字為限）
   研究成果已發表一篇論文（已被Chinese Journal of Physiology被接受但尚未出刊，接受函如附），另一篇為尚未發表之文稿。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）
   在台灣的流行病學研究中，metformin的使用可減少前列腺癌的發生率，另外，胰島素的使用並不會增加前列腺癌的罹患率，關於以上兩點，本研究計畫的結果，皆可為臨床流行病學研究作為基礎研究的驗證。再者，本計畫更進一步發現胰島素對於不表現雄性素接受器前列腺癌細胞生長有促進效果（表現雄性素接受器前列腺癌細胞之生長則無），臨床結果顯示，多數前列腺癌患者仍然表現雄性素接受器或相關活化蛋白，可以解釋胰島素不影響前列腺癌罹患率的原因。因此本研究可解釋流行病學的結果，並且希望可應用在臨床的診斷與治療。